DISINFECTION OF WHITE GRAPE JUICE BY USING CONTINUOUS FLOW UV REACTOR

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for Degree of

MASTER OF SCIENCE

in Food Engineering

by
Zehra KAYA

December 2011
İZMİR

We approve the thesis of **Zehra KAYA**

Assoc. Prof. Dr. Sevcan ÜNLÜTÜRK
Supervisor

Assist. Prof. Dr. Ayşe Handan BAYSAL
Committee Member

Assoc. Prof. Dr. Duygu KIŞLA
Committee Member

14 December 2011

Prof. Dr. Şebnem HARSA
Head of the Department of
Food Engineering

Prof. Dr. R. Tuğrul SENGER
Dean of the Graduate School of
Engineering and Sciences

ACKNOWLEGMENTS

Many persons have contributed either directly or indirectly to my thesis, I would like to mention them and show my thanks in this paper.

Firstly, Iwould like to express my sincere and heartfelt appreciations to my supervisor Assoc. Prof. Dr. Sevcan ÜNLÜTÜRK for her guidance, encouragement and endless support. It is an honour to work with her who is one of the best researchers in İYTE and more than a supervisor for me. I am very grateful to her for giving me opportunity to study with her throughout my master program.

I would like to express my special gratitude to Assist. Prof. Dr. Ayşe Handan BAYSAL who is a member of my thesis committee for her supervision, valuable recommendations in my microbiological experiments. I also want to add my thankfulness to Assoc. Prof. Dr. Duygu KIŞLA from Food Engineering Department at Ege Üniversity for her special kindness in my thesis committee and precious suggestions in my thesis defence.

I especially thank to Research Assistant Mehmet Reşat ATILGAN who is not only my colleague but also has valuable friendship, limitless encouragement and incomparable helpfulness in all my thesis experiments. Additionally, I would like to thank my colleague Merve PELVAN for enjoyable times during experiments and studies in our laboratories.

I am grateful to all my friends in Food Engineering Department of İYTE, close friends and unreplacable persons who are in my life for their favour and giving amusing times for morale.

Before all people, this thesis could not have been undertaken without the support, patience, encouragement and love of my family. Primarily, I wish to thank my dear father İbrahim KAYA who promoted me to be a food engineer and he is also a food engineer. I listen you and always continue my education. I would never been able to achieve without my dear mother Ferah KAYA and her endless advice, thank her for everything. And of course, I am very lucky to have a wonderful sister, my precious, Derya KAYA. I am thankful to her for being the most important supporter and the best friend in my life.

ABSTRACT

DISINFECTION OF WHITE GRAPE JUICE BY USING CONTINUOUS FLOW UV REACTOR

Although continuous flow UV irradiation process is applied to several kind of fruit juices as a non-thermal method, it hasnot treated to white grape juice before. The main objective was to study the effect of commercial UV disinfection system on the spoilage microorganisms in white grape juice. Physical, chemical and optical properties were measured after and before irradiation. Biodosimetric studies were performed by using Bench Top UV apparatus and continuous flow commercial UV reactor.

Bench top UV study showed that 228.96 mJ/cm^2 UV dose provided complete inactivation of *S. cerevisiae* in the pasteurized juice at 0.91 mW/cm^2 UV intensity.. 3.00±0.16 log cfu/ml yeasts and 4.32±0.03 log cfu/ml lactic acid bacteria reduction were detected in naturally fermented juice after 282.24 mJ/cm^2 UV dose. $D_{(10)}$ of yeasts (43.86 mJ/cm^2) were higher than lactic acid bacteria (33.78 mJ/cm^2) because of showing higher resistance to UV light.

In the continuous flow UV reactor, the highest reduction of *S. cerevisiae* was achieved as 3.39±0.044 log cfu/ml at 56.06 mJ/cm^2 UV dose and low flow rate (820 ml/min) in pasteurized juice. However, highest reduction was observed in yeast and lactic acid bacteria count as 1.70±0.028 log cfu/ml and 2.49±0.0031 log cfu/ml respectively at 19.96 mJ/cm^2 UV dose in medium flow rate (1516 ml/min) of fresh squeezed juice. Absorbance, color, and titratable acidity were changed by irradiation. As a result of 13 days shelf life study, no significant increase was seen in spoilage microorganisms after irradiation.

ÖZET

SÜREKLİ AKIM UV REAKTÖR KULLANARAK BEYAZ ÜZÜM SUYUNUN DEZENFEKSİYONU

Sürekli akım UV reaktör sistemi birçok meyve suyu çeşidine ısısal olmayan bir metot olarak uygulanmasına rağmen, bu teknoloji beyaz üzüm suyuna daha önce uygulanmamıştır. Temel amaç, beyaz üzüm suyundaki bozulma mikroorganizmaları üzerine ticari UV dezenfeksiyon sisteminin etkisini çalışmaktır. Işınlama öncesi ve sonrasında fiziksel, kimyasal ve optik özellikler ölçülmüştür. Biyodozimetri çalışmaları kesikli UV aparatı ve sürekli akım ticari UV reaktörde gerçekleştirilmiştir.

Kesikli UV çalışması, 0.91 mW/cm^2'de 228.96 mJ/cm^2 UV dozunun pastörize beyaz üzüm suyundaki *S. Cerevisiae'*nın tamamen inaktivasyonunu sağladığını göstermiştir. 282.24 mJ/cm^2 UV dosajından sonra doğal olarak fermente olmuş meyve suyunda, 3.00±0.16 log cfu/ml maya ve 4.32±0.03 log cfu/ml laktik asit bakterisi azalması saptanmıştır. Mayaların $D_{(10)}$ değeri (43.86 mJ/cm^2) UV ışığına daha direnç göstermelerinden dolayı laktik asit bakterilerinden (33.78 mJ/cm^2) yüksektir.

Sürekli akım UV reaktörde, pastörize meyve suyunda en yüksek *S. cerevisiae* azalması düşük akış hızında (820 ml/min) ve 56.06 mJ/cm^2 dozunda 3.39±0.044 log cfu/ml olarak elde edilmiştir. Bununla birlikte, en yüksek azalma, taze sıkılmış meyve suyunun orda akış hızında, 19.96 mJ/cm^2 UV dozda maya ve laktik asit bakteri sayısında sırası ile 1.70±0.028 log maya; 2.49±0.0031 log cfu/ml laktik asit bakteri olarak ölçülmüştür. Absorbans, renk ve titre edilebilir asitlik ışınlama ile değişmiştir. 13 günlük raf ömrü çalışmasının bir sonucu olarak, bozulma mikroorganizlarında ışınlama sonrası önemli artış görülmemektedir.

This thesis is dedicated to my dear parents, Ibrahim KAYA and Ferah KAYA and my precious sister Derya KAYA who are of great value for me. I love you too much!

TABLE OF CONTENTS

LIST OF FIGURES…….....xi

LIST OF TABLES……..….xv

CHAPTER 1. INTRODUCTION……...…….……..1

CHAPTER 2. LITERATURE REVIEW….……...……...…6

2.1. Fruit Juice…….…...……..…….6

2.1.1. Definition of Fruit Juice…….…….……...…...……6

2.1.2. Composition and Nutrient Content of Fruit Juice……..……..6

2.2. White Grape Juice……..……7

2.2.1. Production of White Grape Juice……..7

2.2.2. Composition and Nutrient Content of Grape Juice…….....11

2.2.3. Health benefits of white grape juice……14

2.2.4. Statistics of grape juice in Turkey…….....15

2.3. Fruit juice quality problems……...18

2.3.1. Spoilage microorganisms and pathogens in fruit juices……..19

2.3.2. Enzymes activities in fruit juices……..…….……..21

2.4. Fruit Juice Preservations……...….22

2.4.1. Thermal Pasteurization……...…….23

2.4.1.1. Technology and Efficiency of the Thermal Pasteurization.....….....23

2.4.1.2. Impacts of Thermal Pasteurization on the Juice Quality Parameters……..….....……..24

2.4.2. Alternative Non-Thermal Preservation Methods……25

2.5. Ultraviolet Light (UV-C) Irradiation…….…28

2.5.1. Ultraviolet (UV) Light…….…28

2.5.2. UV-C Irradiation Mechanisms……29

2.5.3. UV-C Disinfection of Microorganisms…..…….……30

2.5.4. Affecting Factors of UV-C Irradiations in Disinfection……..32

2.5.5. UV Applications in Food Industry……...…….……..33

2.5.6. Advantages & Disadvantages of UV-C Disinfection…..……...34
2.6. Studies of UV-C Irradiation in The Liquid Foods…….35
2.6.1. Bench Top UV Studies (Static UV Systems)……...……...35
2.6.2. Continuous Flow UV Studies……...……...36

CHAPTER 3. MATERIALS AND METHODS…….37
3.1. Raw Materials……...…….……...…37
3.1.1. Pasteurized White Grape Juice……...……....37
3.1.2. Fresh Squeezed White Grape Juice…….....…….…..37
3.2. Physical, Chemical, Optical and Rheological Measurements……..38
3.2.1. pH……..…….……...38
3.2.2. Brix……...……...…38
3.2.3. Titratable acidity……...……...…38
3.2.4. Absorbance……...……...…39
3.2.5. Turbidity……...……...39
3.2.6. Colour……...……..….39
3.2.7. Density……...……..40
3.2.8. Viscosity……...……..……40
3.2.9. Particle size analysis……...……...…….42
3.3. Biodosimetric Studies…….…...42
3.3.1. Target Microorganisms and Cultivation……..…42
3.3.1.1. Inoculation of *Saccharomyces cerevisiae* (Y 139) into Pasteurized White Grape Juice……..……...42
3.3.1.2. Fermentation of Fresh Squeezed White Grape Juice.....…..45
3.3.2. Bench Top UV Study……...…….…47
3.3.2.1. Collimated Beam Apparatus……...……...……..…..47
3.3.2.2. Inactivation Study by means of a bench top UV system.....49
3.3.3. Continuous Flow UV Study……...……50
3.3.3.1. Continuous Flow UV Reactor……..……....50
3.3.3.2. Inactivation Study by means of a continuous flow UV reactor……..…….53
3.3.3.3. Cleaning of the Continuous Flow UV System…..…….....55
3.3.3.3.1. Control of Residual Chlorine After Cleaning Step.....55

3.3.4. Shelf Life Study……56

CHAPTER 4. RESULTS AND DISCUSSION……...…..57
4.1. Physical, Chemical, Optical and Rheological Properties of White Grape Juice Samples……..57
4.1.1. Physical and Chemical Properties…….……...…..57
4.1.2. Optical Properties……..…….…….....…...59
4.1.3. Rheological Properties……..……...……...62
4.1.3.1. Density of the White Grape Juice Samples……..…….62
4.1.3.2. Flow Behaviour of the White Grape Juice Samples..……....63
4.1.3.2.1. Time dependency……...…….....63
4.1.3.2.2. Temperature Dependency……...……65
4.1.3.3. Particle Size Analysis of Fresh Squeezed White Grape Juice……..67
4.2. Biodosimetric results…….……69
4.2.1. Bench Top Biodosimetric Results for White Grape Juice Samples……..69
4.2.1.1. Pasteurized White Grape Juice…...….……...69
4.2.1.2. Fresh Squeezed White Grape Juice……...…….....75
4.2.2. Continuous Flow UV Inactivation Results of White Grape Juice Samples……...…….79
4.2.2.1. Microbiological Results of Pasteurized White Grape Juice……81
4.2.2.2. Microbiological Results of Fresh Squeezed White Grape Juice…….…….89
4.2.2.3. Effect of Continuous Flow UV Inactivation on the Physical, Chemical and Optical Properties of White Grape Juice Samples……....98
4.2.3. Shelf Life Results……..…….....…..……100

CHAPTER 5. CONCLUSION……..102

REFERENCES……..105

APPENDICES

APPENDIX A. MICROSCOBIC APPEARANCE OF NATURAL FLORA IN WHITE GRAPE JUICE……..…..……..…114

APPENDIX B. APPENDIX B. PROPERTIES OF THE WHITE GRAPE JUICE SAMPLES……...……115

APPENDIX C. DATA OF THE CONTINUOUS FLOW UV STUDY……....116

LIST OF FIGURES

Figure **Page**

Figure 2.1. Flow diagram of white grape juice production……....….10
Figure 2.2. Distribution of total fruit juice consumption of Turkey in 2008 (%)…..….17
Figure 2.3. Exportation and Importation of Grape Juice in Turkey from 2005 to 2008 (tone) a. Exportation b. Importation ……....…….....17
Figure 2.4. The electromagnetic spectrum…….....……...…..28
Figure 2.5. DNA structure before and after UV light absorption……....……...…30
Figure 2.6. Typical survival curve of UV exposed microorganisms……....….....32
Figure 3.1. Time dependency of Newtonian fluids…….....….40
Figure 3.2. Dimensions of UL-Y Spindle…….....…41
Figure 3.3. Logarithmic growth curve of the *S. cerevisiae* (Y139) strains in PYM broth (225 rpm, 30˚C)…….....…….44
Figure 3.4. Logaritmic microbial count versus optical density curve of the *S. cerevisiae* (Y139) strains in PYM Broth…….....……...……44
Figure 3.5. Logarithmic growth curve of spoilage microorganisms in the Freshly squeezed white grape juice (100 rpm, 30˚C)……...……46
Figure 3.6. Logaritmic microbial count versus optical density curve of the spoilage microorganisms in the fresh squeezed white grape juice...…….....46
Figure 3.7. Laboratory Scale Closed Bench Top Collimated Beam Ultraviolet System……..48
Figure 3.8. Continuous flow UV reactor system……51
Figure 4.1. Absorbance Versus Dilution Factors Graph of Pasteurized White Grape Juice……60
Figure 4.2. Absorbance Versus Dilution Factors Graph for Fresh Squeezed White Grape Juice (before freezing)……..…60
Figure 4.3. Density values versus temperature curve of pasteurized white grape Juice……..62
Figure 4.4. Density values versus temperature curve of fresh squeezed white grape juice……..63
Figure 4.5. Time dependency of pasteurized white grape juice at a constant shear rate (100 rpm) and different temperatures……...……...64

Figure 4.6. Time dependency of fresh squeezed white grape juice at a constant shear rate (100 rpm) and different temperature……..64
Figure 4.7. Temperature dependency of pasteurized white grape juice at a constant shear rate (100 rpm)…….……..66
Figure 4.8. Temperature dependency of freshly squeezed white grape juice at a constant shear rate (100 rpm)…….……..…66
Figure 4.9. Particle size distrubution of freshly squeezed white grape juice……..68
Figure 4.10. Particle size distribution in all the volume of the fresh squeezed white grape juice…….....68
Figure 4.11. The effect of UV-C dosage on *S.cerevisiae* in the pasteurized white grape juice with 5.50 ± 0.107 log cfu/ml initial microbial load……71
Figure 4.12. UV exposure time curves for S. cerevisiae in the pasteurized white grape juice with 5.5 ±0.107 log cfu/ml initial microbial load. A. Io= 0.91 mW/cm^2, B. Io=0.36 mJ/cm^2 .....…...71
Figure 4.13. The effect of UV-C dosage on *S.cerevisiae* in the pasteurized white grape juice with 7.33 ±0.013 log cfu/ml initial load…….74
Figure 4.14. Exposure time bars for *S.cerevisiae* in the pasteurized white grape juice with 7.33 ±0.013 log cfu/ml initial microbial load. A. Io=0.91 mW/cm^2, B. Io=0.36 mW/cm^2…….....74
Figure 4.15. The effect of UV-C dosage on yeast counts in the fresh squeezed white grape juice at Io = 0.76 mJ/cm^2 (Tray 1), 5.98 ±0.026 log cfu/ml initial microbial load……77
Figure 4.16. The effect of UV-C dosage on lactic acid bacteria counts in the fresh squeezed white grape juice at Io = 0.76 mJ/cm^2 (Tray 1), 7.94 ± 0.039 log cfu/ml initial microbial load……..…..77
Figure 4.17. Exposure time bars for yeasts and lactic acid bacteria in the fresh squeezed white grape juice at the Io = 0.76 mJ/cm^2, 5.98 ±0.026 log cfu/ml initial yeasts and 7.94 ±0.039 log cfu/ml initial lactic acid bacteria……...……..…78
Figure 4.18. Linear model of fresh squeezed white grape juice with the initial yeasts of 5.98 ±0.026 log cfu/ml and lactic acid bacteria of 7.94 ±0.039 log cfu/ml……79

Figure 4.19. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice by using continuous flow UV reactor with 8 cycle at low flow rate (820 ml/min)……..……...……83
Figure 4.20. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice at low flow rate of continuous flow UV reactor system……..83
Figure 4.21. Logarithmic reduction of *S. cerevisiae* in the pasteurized white grape juice by using continuous flow UV reactor with 8 cycle at medium flow rate (1516 ml/min)…….....……..85
Figure 4.22. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice at medium flow rate of continuous flow UV reactor system…….85
Figure 4.23. Logarithmic reduction of *S. cerevisiae* in the pasteurized white grape juice by using continuous flow UV reactor with 8 cycle at high flow rate (2280 ml/min)……..87
Figure 4.24. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice at high flow rate of continuous flow UV reactor system……...87
Figure 4.25. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice in the continuous flow UV reactor system with different flow rates as low (820 ml/min), medium (1520 ml/min) and high flow rate (2280 ml/min)…….88
Figure 4.26. Logarithmic reductions of spoilage microflora in the fresh squezed white grape juice by using continuous flow UV reactor with 8 cycle at low flow rate (774 ml/min)……..……....90
Figure 4.27. Logarithmic reductions of spoilage microflora in the fresh squeezed white grape juice at low flow rate of continuous flow UV reactor system…….90
Figure 4.28. Logarithmic reductions of spoilage microflora in the fresh squezed white grape juice by using continuous flow UV reactor with 8 cycle at medium flow rate (1512 ml/min)……....92
Figure 4.29. Logarithmic reductions of spoilage microflora in the fresh squeezed white grape juice at medium flow rate of continuous flow UV reactor system……..…92
Figure 4.30. Logarithmic reductions of spoilage microflora in the fresh squezed white grape juice by using continuous flow UV reactor with 8 cycle at high flow rate (2085 ml/min)……..94

Figure 4.31. Logarithmic reductions of spoilage microflora in the fresh squeezed white grape juice at high flow rate of continuous flow UV reactor system……...….94
Figure 4.32. Logaritmic reductions of spoilage microflora in the fresh squeezed white grape juice in the continuous flow UV reactor system with different flow rates A. yeasts, B. lactic acid bacteria…….95

LIST OF TABLES

Table **Page**

Table 2.1. Composition of the grape juice……...11
Table 2.2. Amounts of fruits and vegetables that processed to the juice in Turkey 2000-08 (kiloton)…….....16
Table 2.3. Juice Safety and Deterioration Hazards……..18
Table 2.4. Fruit juices – associated food poisoning outbreaks……..…….....20
Table 2.5. Advantages and Disadvantages of Minimally Processed Juice……..26
Table 2.6. New non-thermal food processing methods……...27
Table 2.7. UV dose requirement of some microorganisms for complete inactivation…….31
Table 3.1. Options of Continuous Flow UV Reactor……..52
Table 3.2. Flow parameters of white grape juice samples at different frequency Levels……..54
Table 4.1. Physical and Chemical Properties of White Grape Juice Samples…….58
Table 4.2. Optical properties of White Grape Juice Samples……..59
Table 4.3. Log reductions of pasteurized white grape juice at different microbial load (5.5 ±0.107 log cfu/ml and 7.33 ±0.013 log cfu/ml) with two different intensities as Io=0.91 mW/cm^2 (Iavg= 0.636 mW/cm^2) and Io=0.36 mW/cm^2 (Iavg=0.252 mW/cm^2)…..……...69
Table 4.4. Decimal reduction dose ($D_{(10)}$) and inactivation rate constants (k) of pasteurized white grape juice samples in the Bench Top UV Study……73
Table 4.5. Log reductions of spoilage microorganisms in the fresh squeezed white grape juice at a constant UV-C intensity of Io=0.76 mW/cm^2…….....75
Table 4.6. Decimal reduction dose ($D_{(10)}$) and inactivation rate constants (k) of fresh squeezed white grape juice samples in the Bench Top UV Study.....78
Table 4.7. Flow and UV parameters for pasteurized and fresh squeezed white grape juice.....……80
Table 4.8. Decimal reduction dose ($D_{(10)}$) and inactivation rate constants (k) of pasteurized white grape juice samples in the continuous flow UV reactor system.....88

Table 4.9. Decimal reduction dose ($D_{(10)}$) and inactivation rate constants (k) of fresh squeezed white grape juice samples in the continuous flow UV reactor system.....97
Table 4.10. Physical, chemical, and optical properties of pasteurized white grape juice samples before and after continuous flow UV irradiation…....99
Table 4.11. Physical, chemical, and optical properties of fresh squeezed white grape juice samples before and after continuous flow UV irradiation.....100
Table 4.12. Microbiological results of fresh squeezed white grape juice during 13 days.....101

CHAPTER 1

INTRODUCTION

Fruit juice is defined as the unfermented but fermentable liquid produced from well, ripe and fresh fruit or fruit obtained in sound conditions according to restrictions of the Codex Alimentarious Commission. Fruit juice is directly obtained from fruits by some mechanical treatments and have the same characteristic properties of the fruits that are derived from such as physical, chemical, organoleptical and nutritional properties (World Health Organization and Food and Agriculture Organization of the United Nations, 2005). Fruit juice contains many sugars, vitamins, minerals, organic acids, amino acids and fenolic matters (Cemeroğlu, 2004). Because of their nutritional properties, the consumer demand on the consumption of fruit juices has been rising in the last decades (Bates, et al. 2001).

White grape juice is one of the low acid fruit juice that is produced for consumption directly. It is physically extracted from mature and suitable grapes, and preserved by physical methods. It can be in the clarified or cloudy form (World Health Organization and Food and Agriculture Organization of the United Nations, 1981). The composition of the white grape juice consists of many compounds such as carbohydrates, organic acids and proteins. It is also a good source of vitamins (A, B and C) and minerals (Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Publication, 2006). White grape juice has various health benefits due to its antioxidant, anti-carcinogenic, anti-coagulant, anti-inflammatory properties (Perfect Berry 2007). It is recommended as "the baby's first juice" due to easy digestibility properties (Welch's international, 2008). Given these facts, the consumption rate of the grape juice has an increasing trend in Turkey (Meyed, 2008).

The major problem is originated from the consumption of contaminated and unpasteurized fruit juices by pathogenic microorganisms surviving in the low acidic conditions. For instance, outbreaks of *Esherichia coli* O157:H7, *Salmonella enterica, Listeria monocytogenes* are associated with the consumption of unpasteurized fruit juice resulted in fatalities in the last decade (Cook, et al. 1998; Gabriel and Nakano, 2009; Pathanibul, et al. 2009). In addition to pathogen microorganisms, spoilage

microorganisms such as yeasts, moulds and acid-tolerant bacteria that can occur naturally in fruit juices have also led to off-flavors and off-odors, reduction in quality and color change of the juices (Tournas, et al. 2006). The Center for Disease Control and Prevention (CDC) reported 21 outbreaks originated from fruit juice consumption from 1995 to 2005 and 1.366 relevant illnesses were recorded in this period (Vojdani, et al. 2008). In order to minimise outbreaks induced from fruit juice consumption, the U.S. Food and Drug Administration (FDA) enforced the control of fruit juice processing based on regulations of Hazard Analysis Critical Control Point (HACCP) programme (Federal Register [FR], 2001). According to the related regulation (21 CFR 110), 5 log reduction in the number of target microorganism is found to be necessary for fruit juice pasteurization (USFDA, 2001).

Conventional thermal pasteurization is an effective technique used to destroy spoilage and pathogenic microorganisms and inactivate enzymes in the fruit juices (Rajashekhara, et al. 2000; Pathanibul, et al. 2009; Igual, et al. 2010; Valappil, et al. 2009). Thermal pasteurization in fruit juice manufacturing is applied mainly between 60 and 100 ˚C for a number of seconds at the pH equal to or less than 4.5 (Rivas, et al. 2006). For instance ,white grape juice is pasteurized at 90-95˚C for 15-30 s (Cemeroğlu, 2004). Although this thermal process provides an effective pasteurization, long and stable shelf life of the fruit juice, several sensorial and nutritional quality problems such as permanent loss of flavour and taste, degradation of nutrients and undesirable browning reactions emerged from heating (Walkling-Ribeiro, et al. 2008; Elez-Martinez, et al. 2005; Garde-Cerdán, et al. 2007; Charles-Rodriguez, et al. 2007). In order to prevent this harmful effects of the thermal pasteurization, non-thermal processes are investigated as an alternative preservation method for more fresh, wholesome and healthful fruit juices (Rivas, et al. 2006; Schenk, et al. 2011; Noci, et al. 2007). Since the temperature is kept lower than thermal pasteurization, the adverse effects of heat on the juice properties are prohibited (Raso and Barbosa-Cánovas, 2003).

Some of these non-thermal alternative methods are high hydrostatic pressure (HHP), pulsed electric field (PEF), oscillating magnetic fields (OMF), high-intensity ultrasound, ultraviolet light (UV), aseptic packaging, addition of antimicrobials (Butz and Tauscher, 2002; Bates, et al. 2001). These methods may also have some unfavourable impact on the food quality. For example, Noci, et al. (2008) applied PEF to fresh apple juice and a slight discoloration was detected. In another study, Vitamin A content in the orange juice was decreased slightly by raising PEF treatment times

(Cortés et al. 2006). Tiwari et al. (2008) reported ultrasonication effects on the fresh squeezed orange juice. According to their study, brix, pH and titradable acidity values of the orange juice were not changed, however, color parameters (L*, a*, b*) and browning index of the juice were enhanced with ultrasonic treatment. Talcott et al. (2003) showed higher ascorbic acid losses than thermal pasteurization in muscadine grape juice when they applied high hydrostatic pressure (HHP) (Talcott, et al. 2003).

It is shown that UV-C (short wave ultraviolet light) light between 220 and 300 nm in the electromagnetic spectrum has a lethal impact on microorganisms such as bacteria, yeasts, moulds and viruses (Gayan, et al. 2011). FDA approved the use of UV-C irradiation as an alternative pasteurization method for fresh juices (US FDA, 2000). This process does not produce chemical residues and does not change the odor, flavour, color and pH too much. In addition, the process is practical, safe and the equipment is not costly. The technique is very effective against many microorganisms (Bintsis, et al. 2000; Murakami, et al. 2006). The most efficient germicidal UV light is used at 254 nm and applied for disinfection purposes on the surface of fresh products and shell eggs, in water, liquid foods such as fruit juices, liquid egg products, milk and sugar syrup (Beltrán and Cánovas, 2005; Tran and Farid, 2004; Gómez, et al. 2010). The mechanism of UV inactivation is based on the absorption of UV light by nucleic acids of microorganisms causing breakage in bonds of their DNA and crosslinking of adjacent pyrimidine bases on the same DNA strand. These dimers protect cells from replicating and provide inactivation of microorganisms (Kouchma, et al. 2009).

There are a number of studies related to the application of UV-C light irradiation in the literature. In these studies, the samples first inoculated with a certain number of microorganisms cultivated in a lab environment then exposed to UVC irradiation. For example, Unluturk et al. (2010) studied the inactivation effect of UV-C irradiation on different microorganisms inoculated into liquid egg white by using collimated beam apparatus. They indicated that *E. coli* O157:H7 (NCTC12900, ATCC 8739) strain was more sensitive to UV irradiation than *E. coli* K-12 (ATCC 25253) and *Listeria innocua* (NRRL B33314). They reported 1.403 log reduction after exposing liquid egg white samples spiked with this microorganism for 20 min at 26.44 mj/cm^2 UV dose. Similarly, Ngadi et al. (2003) studied the inactivation of *E. coli* (O157:H7) in the apple juice by using collimated beam apparatus. They achieved 4.8 log reduction for 1 mm fluid depth at 6.5 mWmin/cm^2 UV dose in the apple juice. On the other hand, the reduction was

only 3.5 log when the juice depth was 3.5 mm at the same UV dose. They realized that increasing the fluid depth resulted in less UV inactivation.

Cantwell and Hofmann (2008) reported 3.4 log reduction for the indigenous coliform bacteria found in unfiltered surface water samples after applying 20 mj/cm^2 UV dose using a flow-through UV reactor system. Keyser et al. (2008) used PureUV turbulent flow system for inactivation of microorganisms in the clear apple juice. After exposure of 234 mj/cm^2 UV dose, they achieved 3.5 log and 3 log reduction in the number of aerobic bacteria, and yeast and mould counts, respectively. Similar continuous flow UV study was performed by Tran and Farid (2004). They measured aerobic, yeasts and mould counts in fresh squeezed orange juice after exposing to UV light using a thin film UV reactor. It was found that 87 and 119 mj/cm^2 UV dose were required for one decimal reduction of aerobic bacteria, and yeasts and moulds in the orange juice, respectively. Koutchma et al. (2006) performed UV inactivation of E.coli K12 study in apple cider using Cidersure multiple-lamp laminar flow and Aquionics multiple-lamp turbulent flow UV reactor systems. Their paper demonstrated that the effect of different flow type on the UV inactivation of E.coli K12 by comparing UV decimal reduction dose. Although Aquionics UV reactor system required UV dose ranging between 90 and 150 mj/cm^2 at 1250 ml/s flow rate for one decimal reduction in the apple cider, UV dose was ranging from 18.8 to 25.1 mj/cm^2 for thin film Cidersure UV reactor at 56.8 ml/s for the same decimal reduction. It was understood from this study that turbulent flow conditions were not very influential for high turbid fruit juice such as apple cider.

Comparing logarithmic reductions achieved in liquid foods by means of bench top and continuous flow UV reactors, it was demonstrated that more effective results were obtained in bench top studies due to having low penetration depth. Thus, further studies by means of continuous flow UV reactor are necessary to be performed for better comprehension of the system effectiveness especially on the natural flora of the juice.

The objective of this work is;

- to test the performance of a pilot scale commercial continuous flow UV reactor on the inactivation of spoilage microorganism of fresh squeezed white grape juice and pasteurized white grape juice inoculated with *Saccromyces cerevisiae* at different flow rates.

- to evaluate the effect of UV-C irradiation on the physical, chemical, optical properties before and after treatment.
- UV dose determination of microorganisms in the fresh squeezed and pasteurized white grape juice by Bench Top UV studies.

CHAPTER 2

LITERATURE REVIEW

2.1. Fruit Juice

2.1.1. Definition of Fruit Juice

Fruit juice is defined as ‘a beverage that is not reconstituted, including no additive, composed 100% of fruits’ in Turkish Food Legislation (Cemeroğlu, 2004).

Codex Alimentarious Commission of Turkey identifies fruit juice as the unfermented but fermentable liquid that is made from well, fully grown, fresh fruits or fruits harvested from suitable conditions. Fruit juice is directly processed by feasible mechanical ways and has the physical, chemical, organoleptical and nutritional properties of fruits that is obtained from. Some of the fruit juice is treated with their seeds, pips or peels which transferres few parts of constituents to the juice. The juice may be produced as clear or cloudy and from single type or mixed fruits (World Health Organization and Food and Agriculture Organization of the United Nations, 2005).

2.1.2. Composition and Nutrient Content of Fruit Juice

Fruit juice maintains almost all properties of fruit which are obtained. Regardless what kind of fruit that is produced from, the main ingredient of the fruit juice is water. Therefore, a great majority of water soluble components such as sugars, acids, free amino acids, minerals, water-soluble vitamins and fenolic matters are moved into the fruit juice content; however, water insoluble or very little soluble substances such as polysaccharides (cellulose and pectic matters), lipids and carotenoids are not transferred to the juice (Cemeroğlu, 2004). Fruit juice has fat or protein in small amount, so these are not attended as a juice component.

Following the water, the most available components found in the fruit juice are carbohydrates i.e. fructose, glucose and saccharose. Although glucose and fructose are

usually found in the fruit juice, the amount of saccharose is variable in terms of fruit kinds (Artık and Velioğlu, 1992). Organic acids in fruits are important to detect maturity of the fruits, adulteration and spoilage in juices.. However, they are also utilized as additive in the fruit juice manufacturing (Soyer, et al. 2003). Non-volatile organic acids such as malic, tartaric and citric acids are the flavour components of the fruit juice and act a great role on colour and stability of the juice (Cunha, et al. 2002). Further, volatile acids like formic and acetic acid increases in mashing of the juice. Free amino acids soluble in water are naturally transferred to the fruit juice during processing (Artık and Velioğlu, 1992). Although amino acids are present in a little quantity in the fruit juices, they are essential because of their reactions with reducing sugar in non-enzymatic browning of the juice (Maillard reaction) (Ting and Deszyck, 1960). Although products of this reaction provide antoxidant and antimicrobial effect, browning causes the loss of quality of the fruit juices (Burdurlu and Karadeniz, 2003). In addition to these components, potassium, magnesium, calcium, sodium, zinc, copper and iron are the main minerals in fruit juice (Winiarska-Mieczan and Nowak, 2008).

Vitamin A, C, E are not only the essential vitamins found in fruit juice but also show antioxidant properties that provide cardiovascular health, anticarcinogenic effect, anti-aging and protective effect on Alzheimer disease (Zulueta, et al. 2007). Carotenoids showing provitamin A activity causes antioxidant activity in the fruit juice, as well (Gardner, et al. 2000; Pena, et al. 2011). Besides these nutrients, flavanoids are important phenolic elements that are present in the juice because of impressing blood circulation, providing more conductance and flexibility of the capillaries (Ashurst, 2005).

2.2. White Grape Juice

2.2.1. Production of White Grape Juice

Basic steps in producing clear white grape juice are shown in Figure 2.1. Different methods may be carried out in processing of grape juice, these methods can be changed in terms of variety and structure of grapes, kind of juice that will be produced. The major difference between white and red grape juice production is in pressing line. While hot pressing and skin maceration (extruded red grapes are kept in a tank at 60-

63 ˚C adding with pectolytic enzyme) are applied to the red grapes to pass color pigments of the skin to the juice, the white grape juice processing has only cold pressing to prevent its light color and enzymes are applied during clarification and filtration steps (Anlı, 2006; Somogyi, et al. 1996).

Production of white grape juice is started with harvesting the fruits. Grapes are mechanically harvested for better fruit quality. The kind of machine, variety of grape, production system, temperature of harvesting, postharvest handling treatments are major factors influencing quality of the grapes (Bates, et al. 2001). Grapes should have sufficient maturity to obtain high quality juice, temperature should be controlled to protect from enzyme and microbial activity in storage, handling should be achieved properly in the juice production (Somogyi, et al. 1996).

Mechanically harvested grapes are washed in a belt washer to remove foreign substances from the grapes (Cemeroğlu, 2004). Grapes are crushed between cylinders after washing step. Stems are not separated in order to improve pressing yield. This does not affect the flavour of the juice, because cold pressing is applied to the white grapes and bitter, astringent taste of stems are not passed to the juice (Cruess, 1938; Cemeroğlu, 2004). However, in some cases, stems of the grapes are splitted through a specially made up stemmer before crushing, also partially smashed by bumping effect of stemmer (Jacobs, 1951; Cemeroğlu, 2004). Crushed white grapes are transferred to dripping tanks, after that, 30%- 40% of the white grape juice is extracted before pressing. Remaining mash of white grapes is pressed without heating, if cloudy white grape juice is acquired (Cemeroğlu, 2004).

Pressed white grape juice has many insoluble solids, which are removed by centrifugation, rotary vacuum filtration or pressure leaf filtration (Somogyi, et al. 1996). Although clear white grape juice is obtained soon after centrugation, argols and thermolabil proteins in the juice composition can cause cloudiness during storage. Depectinization and detartarization steps are two main operations to obtain stabilized white grape juice. Pectolitic enzymes are added to the juice and provide breaking up pectins of the juice (depectinization). Therefore, charged proteins are free and get over pectins that cover them. After depectinization, juice is clarified by adding clarification agents such as bentonit, kieselsol and gelatine. Bentonit has negative charge and make flocculation with positive charge of the protein particles in the juice. Similarly, gelatine has positive charge and sedimentation occur by combining positive charge of gelatine with negative charge of fenolics to prevent the juice from astringent and bitter taste of

fenolic matters such as tannins. However, gelatine does not make flocculation and increases turbidity of the juice above 40 ˚C. In this case, Kieselsol are added to the juice, it raises flocculation effect of gelatine with fenolics and linked with gelatine to protect juice from gelatine residues after clarification. Clarified and stabilized juice is filtered in kieselguhr filters and transferred to the KZE tanks (Kurzzeit- erhitzung means HTST) (Cemeroğlu, 2004).

After protein stabilization, argols that are the crystal form of potassium hydrogen tartarates ($KHC_4H_4O_6$) can make precipitation like proteins in the juice in time. Thus, argols are the major important problems and stabilization can be provided in processing of the juice by adding specific polymers, removing tartaric acids from the juice or holding tanks at cold temperature by shaking regularly (-2 or 0 ˚C). In the juice industry, the most general usage for removing tartarates is a cold storage of clarified and filtered juice in holding KZE tanks, argols sediments from the juice at cold temperature (detartarization) and they are removed from the juice by re-filtering before packaging (Cemeroğlu, 2004). Additionally, it is indicated that maturity, total acidity and storage time of the juice are essential for detartarization step in the juice processing (Bates, et al. 2001).

After potassium hydrogen tartarate stabilization, filtered juice is pasteurized at 90-95 ˚C for 15-30 s. After pasteurization, juice is filled to the cartoon packages aseptically. In another way, juice in KZE tanks is filled to the glass bottles and then pasteurized (Cemeroğlu, 2004).

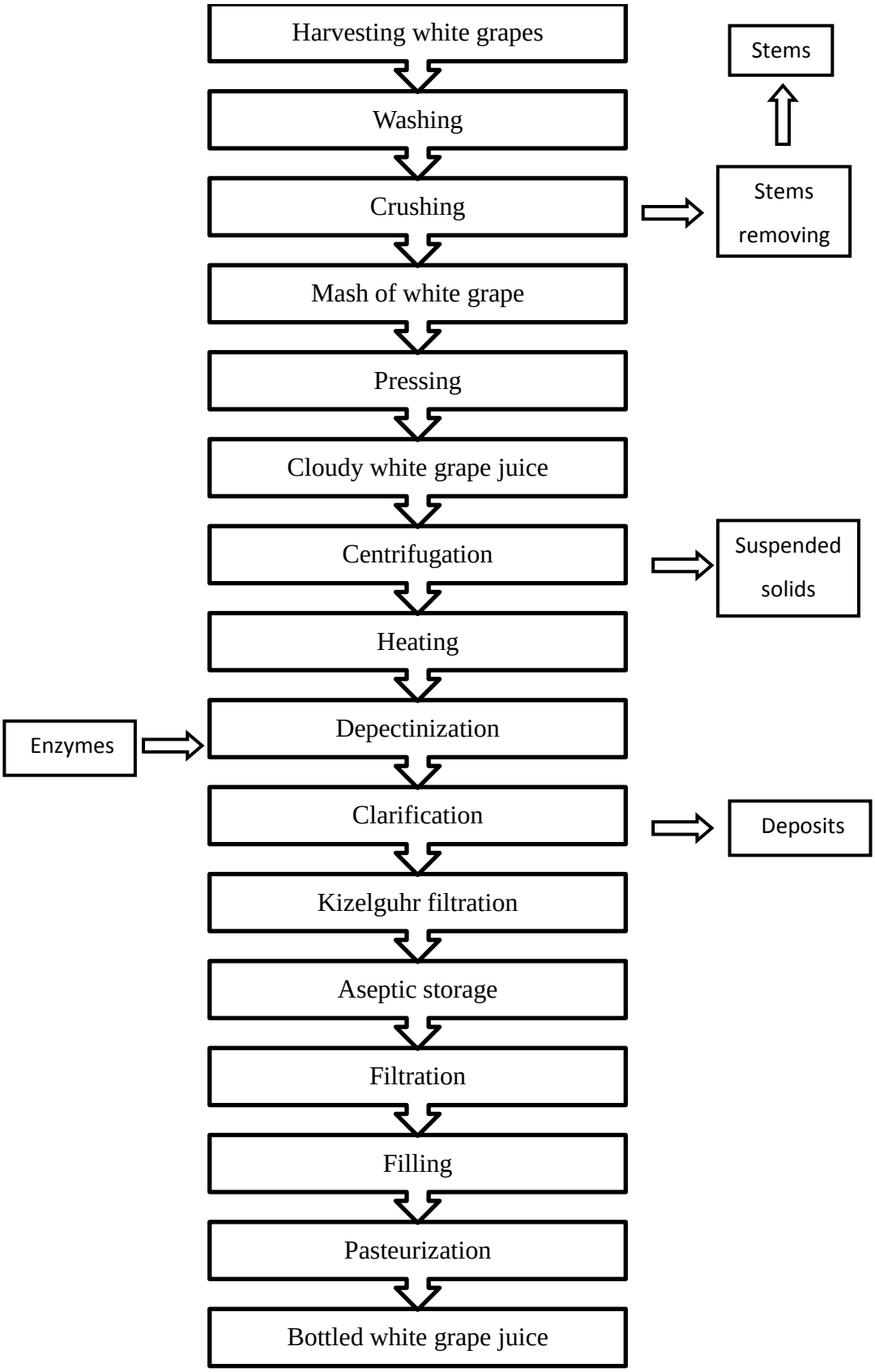


Figure 2.1. Flow diagram of white grape juice production.
(Source: Cemeroğlu, 2004)

2.2.2. Composition and Nutrient Content of Grape Juice

The demand of the grape juice has increased rapidly because of their rich nutritive value and a number of benefits to the human health. Composition of grape juice is listed in Table 2.1. It is not very different from the grapes, the only difference is that lies in the oil and crude fiber content presented in the seed that is removed from grapes during processing (Peterson, 1980; Somogyi, et al. 1996; Bates, et al. 2001). The composition of the grapes is altered during ripening of the fruit and changed from area to area, according to soil, location and climatic situations (Somogyi, et al. 1996). The quality of the juice depends on the amount of sugar, acid ingredient and flavour materials such as methyl anthranilate and other volatiles, tannins and colour substances (Bates, et al. 2001).

Table 2.1. Composition of the grape juice (Source: Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Publication, 2006).

Grape juice (100g)	
Energy (kcal)	66
Water (g)	82.9
Carbohydrates (g)	16.6
Protein (g)	0.2
Fat (g)	0.0
Ash (g)	0.3
Calcium (mg)	11
Phosphore (mg)	12
Iron (mg)	0.3
Sodium (mg)	2
Potassium (mg)	116
Thiamin (mg)	0.04
Riboflavin (mg)	0.02
Niasin (mg)	0.2
Ascorbic acid (mg)	1.7

The major part of the grape juice is water (82.9%) which is the same as the composition of all fruit juices (Republic of Turkey Ministry of Agriculture and Rural

Affairs General Directorate of Publication, 2006). Carbohydrates such as glucose and fructose are the main components of grape juice. However, some variety of the grapes contains sucrose in the amount equal to one third of the total sugar content in the juice (Somogyi, et al. 1996). Welch Juice company expressed that white grape juice has an equal amounts of glucose and fructose and contains no sorbitol in its composition. This balance of sugars causes easy digestion by infants, prevent gas, distension, diarrhea, and insomnia problems affecting co-mechanisms. If the amount of fructose is higher than glucose, the malabsorption of the fructose and digestion problems occur. Additionally, sorbitol in the some fruit juice has indigestible effect in the metabolisms of toddlers and infants (Perman, 1996).

Proteins are part of the nutrient content of grape juice and wines. Protein stabilization is provided by adding bentonite and removed during processing after precipitation (Zoecklein, 1991). Likewise, grape juice contains many minerals such as sodium, potassium, calcium, phosphorus, iron, copper, manganese that have supplementary to the health (Somogyi, et al. 1996). Potassium is a major mineral in the grapes and an increase in the amount of potassium causes pH level of the fruits to increase (Peterson, 1980). Sodium, potassium, calcium and magnesium are found in higher concentration than iron (Feng, et al. 1997).

Grape juice is a substantial fruit juice in respect to vitamin B content. Niasin (B_3), pantothenic acid (B_5), thiamine (B_1), folic acid (B_9), pyridoxine hydrochloride (B_6), biotin (B_7), choline, ascorbic acid and minor amount of riboflavin (B_2) and cobalamine (B_{12}) are vitamins in the grape juice (Somogyi, et al. 1996; Peterson, 1980).

The major acid of the grape juice is tartaric acid. Tartaric acid may be present as in the free form or as salt (Somogyi, et al. 1996). In addition to this, malic acid is another main organic acid, others are citric acid, trace amount of lactic, isocitric and salicylic acids (Souci, et al. 1989/1990). It is indicated that tartaric acid along with malic acid is composed of about 90% of the total organic acid composition of grape juice. Organic acids are essential determinants of fruit maturity. During growth, total acid matter is at the highest level, but it falls off in the ripening of the grape (Soyer, et al. 2003). In conjuction with this, acidity of the grapes influences the quality of the juice. If the acidity increases above 0.85 % of the regular level, the grape juice possesses a very sharp taste (Somogyi, et al. 1996). Additionally, organic acids are also added to fruit juices as acidifiers to protect it from spoilage of the juice (Soyer, et al. 2003).

The quality of grape juice is based on acidity, the amount of sugar and flavour substances such as methyl anthranilate, tannins and colour pigments (Bates, et al. 2001). It is also reported that aromatic compounds including acids, alcohols, aldehydes, ketones and esters in the grapes are also effecting the quality of grape juice (Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Publication, 2006). Methyl anthranilate is an aromatic volatile compound responsible for odour of certain grape species (Somogyi, et al. 1996). Tannins are polyphenolic compounds that are present in seeds and skin of the grapes such as seed tannins and skin tannins (Cosme, et al. 2009). They aggregate with proteins and bloke enzyme activity and cause more enzyme addition in depectinization step (Cemeroğlu, 2004).

It is known that grapes are rich in polyphenols. Catechin, epicatechin, epicatechingallate, caftaric acid, coutaric acid (cis- and trans- form) are identified in the white grape species in the literature (Borbalan, et al. 2003). These phenolic matters are also found in grape juice products (Dani, et al. 2007). It is indicated that phenolic substances are effective in the flavour, taste and color of the foods. Additionally, chlorophyll and carotenes that are present in the grapes are responsible color pigments in the white grape juice (Somogyi, et al. 1996).

Recently, trans-resveratrol (3,5,4'-trans-trihydroxy-stilbene) which is a polyphenol is detected in the grape juice because of its antioxidant and anticarcinogen properties. It is a phytoalexin and produced by defence mechanism of plants during unfavourable situations or pathogen invasion. Although the amount of resveratrol in the grapes has a nominal value, it changes depending on the grape cultivar, attacks to grape and environment (Anlı, 2006; Gonzales-Barrio, 2009).

The important enzymes in the grape juice composition are invertase, oxidase, protopectinase and pectase (Somogyi, et al. 1996). Polyphenoloxidase (PPO) and peroxidise (POD) are undesired enzymes and should be inactivated in the white grape juice in order to prevent color and aroma disorders of the juice (Marselles-Fontanet and Martin-Belloso, 2007). Pectolytic enzymes are added to the juice during clarification to break colloidal pectins and release the proteins (Cemeroğlu, 2004).

2.2.3. Health Benefits of White Grape Juice

Generally, red and white grape juices are healthy juices in terms of vitamin, mineral phenols, resveratrol content and other compounds that are present in their composition. The daily consumption of white grapes provides sufficient energy for the body along with other fruits (The perfect berry, 2007). In relation to this, needed energy is supplied soon after drinking grape juice (Juicing for health, 2005-11).

Red grape juice has a slightly more advantages than white grape juice because during maceration processing, the heating is applied providing passage of antioxidant phenols in the skin and seeds to the juice. Phenolic substances have an effect on the color, flavour and aroma of foods. Although they exist as a minor amount in the fruits, they have essential biological activity (Anlı, 2006). Phenolics in the grape juice prevent eggragating of platelet, protect LDL (low density lipoprotein) from oxidation and exclude atherosclerosis and heart diseases (Dani, et al. 2007).

Resveratrol (3,5,4' -trihydroxy-stilbene) that occurs as a result of the injury of the fruits is a phenolic compound. However, the white grape juice does not contain a great amount of resveratrol which has antioxidant, anti-carcinogen, anti-inflammatuary characteristics and prevents cardiovascular diseases. In addition, resveratrol inhibites atherosclerosis by preventing the low density lipoprotein oxidation (Fremont, 2000).

Grapes include many organic acids such as tartaric, malic and citric acid and they act a medical role on the human health. Minerals in the grape juice such as potassium, calcium, sodium, phosphore help purification of the livers. While, dark grape juice has iron blocking chemicals, white grape juice gets rid of fatigue by supplying iron to the body, especially for women that have iron defiency (Healthy Menu Mailer, 2005-07; Organic Facts, 2011). Addition to these benefits, skin protection is provided by grape juice consumption due to high vitamin C content (Juicing for health, 2005-11).

White grape juice helps digestion of the sugars by infants and toddlers because it contains an equal amount of fructose and glycose (1:1 ratio) and no sorbitol (hardly digested sugar like compound) in its composition (Welch International, 2008). Welchs juice company indicated that babies and young children up to five years old cannot absorbe fruit juices that have high fructose:glycose ratio and sorbitol as well. Thus, by

consumption of white grape juice, fructose can be absorbed easily, inhibit carbohydrate malabsorption causing diarrhea, gas problems, crying, insomnia.

As a summary, advantages of white grape juice to the human health are listed under several major titles. These are;

- *Antioxidant:* phenolic compounds have antioxidant properties, prevent low density lipoproteins from being oxidated
- *Anti-aging:* antioxidants have an effect on reduction of aging
- *Anti-carcinogenic:* high content of antioxidant compounds such as resveratrol inhibite cancer
- *Cardiovascular health protection:* resveratrol in the grapes is a good cleaner of arterial deposits
- *Anti-coagulant:* help blood circulation and prevent blood flocculation in the body
- *Anti-inflammatory:* decrease problems such as asthma, rheumatism and gout
- *Anti-bacterial/viral:* protect from infections
- *Fever preventer:* supply energy for the body and decrease fatigue
- *Gastric health provider:* prevent indigestion, relieve stomach, preferred as a laxative juice
- *Kidney cleaner:* reduce the acidity of uric acid, eliminate acids from the system
- *Liver cleaner:* play a role on liver purification with the minerals in it.
- *Bowel cleaner:* reduce constipation being a laxative
- *Provide brain, skin, eye health* (The perfect berry, 2007; Juicing-for-health, 2005-11; Healthy Menu Mailer, 2005-07; Organic facts, 2011).

2.2.4. Statistics of Grape Juice in Turkey

Grape juice consumption has been rising in the world due to progressively introducing health advantages. Italy, Spain, Argentina, USA and France are significant manufacturers and exporters in the market of grape juice in the world. Additionally, USA, Germany, Italy, Canada and Japan are major importers. USA is the most important importer of grape juice in the world and it produces grape juice (Anlı, 2006).

There is an increasing demand for fruit juice processing in Turkey. Table 2.2. indicates the amount of fruits and vegetables that are processed to the juice in Turkey from 2000 to 2008. According to this table, the amount of fruits and vegetables processed to juice showed 78% increase in 2008 compared to 2000. Besides, grapes began to be used for the juice production in 2005. Although 10.9 kiloton grapes were used for the grape juice in 2005, these amounts increased to 18.3 and 16.9 kilotons in 2007 and 2008, respectively. In other words, while grapes processed for the juice constitute 1.7% of the total fruits in 2005, they enhanced to 2.2% in 2008 (Meyed, 2008).

Table 2.2. Amounts of fruits and vegetables that processed to the juice in Turkey 2000-08 (kiloton) (Source: Meyed, 2008).

FRUITS	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
Cherry	20.4	28.2	9.9	54.7	35.7	37.1	52.2	72.6	54.6
Apricot	26.7	37.2	13.9	34.8	24.8	30.8	36.1	38.2	74.9
Peach	44.8	31.5	26.2	51.5	30.2	75.9	65.3	90.1	118.8
Apple	311.5	272.9	244.5	341.5	338	409.2	282.9	356.8	333.8
Orange	22.9	12.6	31.7	28.3	46.2	33.1	37.8	53.3	63.9
Pomegranate	-	-	-	-	-	17.6	46.6	57.5	49.5
Carrot	-	-	-	-	-	-	-	30.6	30.7
Grape	**-**	**-**	**-**	**-**	**-**	**10.9**	**8.4**	**18.3**	**16.9**
Strawberry	-	-	-	-	-	-	-	4.1	7.7
Grape fruit	-	-	-	-	-	-	-	-	5.5
Quince	-	-	-	-	-	-	-	7.5	4.5
Tomato	-	-	-	-	-	4.6	4.9	3.9	4.4
Lemon	-	-	-	-	-	-	-	-	2.7
Others	6.8	6	19.3	10.5	16.9	10.2	47.9	4.3	3.2
Total	**433.1**	**388.4**	**345.5**	**521.3**	**491.8**	**629.4**	**582.1**	**737.2**	**771.1**

Grape juice consumption ratio of Turkey in 2008 developed when it is compared to 2007. In 2007, grape juice consumption was 7.13 % of the total fruit juice consumption. However, this percentage was increased to 8.13 % in 2008 (Figure 2.2). Other than these estimations, the total fruit juice consumption was 52.8 million liters in 2008 and the total grape juice consumption was 4.3 million liters (Meyed, 2008).

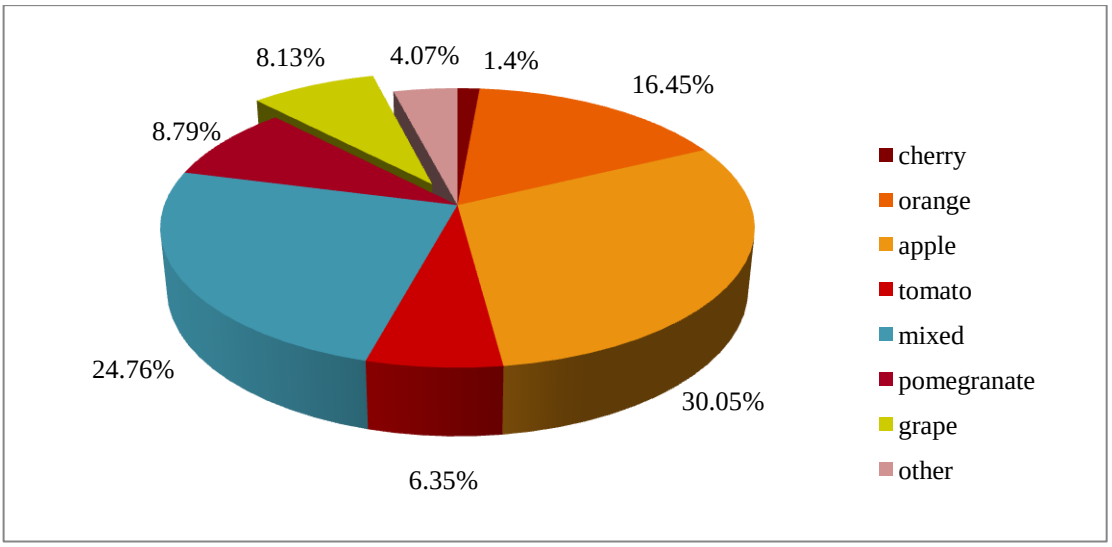
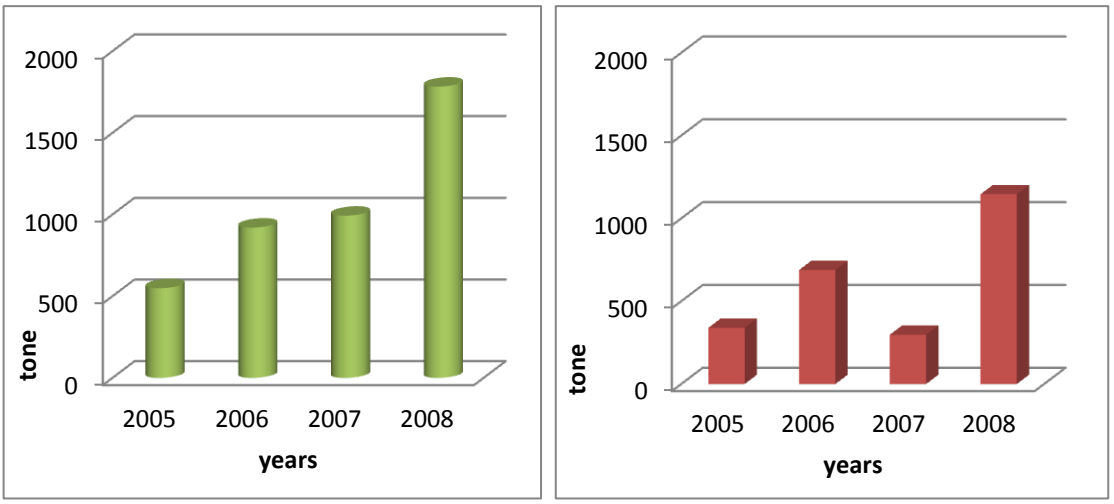


Figure 2.2. Distribution of total fruit juice consumption of Turkey in 2008 (%). (Source: Meyed, 2008)



(a) (b)

Figure 2.3. Exportation and importation of grape juice in Turkey from 2005 to 2008 (tone). a. Exportation b. Importation (Source: Meyed, 2008).

Grape juice importation and exportation of Turkey from 2005 to 2008 were represented in Figure 2.3. Despite the fact that importation of grape juice did not increase properly, exportation of grape juice was growing from year to year. Though 548 tonnes of grape juice were exported to overseas in 2005, 2008 statistics showed that this amount was reached to 1783 tonnes increasingly.

2.3. Fruit Juice Quality Problems

Unprocessed fruit juice is a product that tend to spoilage more than whole fruits because its components are more in contact with air and more accessible for microorganisms. Therefore, it can be spoiled and deteriorated by microorganisms, enzymes, chemical and physical factors. Table 2.3. marked general deterioration factors in the fruit juices. Among these deterioration factors, microbial and enzymatic spoilage are the main causes and several inhibitive methods must be applied to preserve the juice. These quality problems were explained in details section 2.3.1. and 2.3.2. (Bates, et al. 2001).

Other quality losses are dissolved oxygen, metal cautions and other components that change taste, aroma and color in the fruit juice according to the Table 2.3. Non-enzymatic Maillard reaction between reducing sugars and amines in the fruit juice produces brown pigments and leads to undesirable color in the juice. Additionally, protecting juice from pesticide residues, filtration of undissolved particles and avoiding metals during juice processing are essential rules in order to produce qualified fruit juice (Bates, et al. 2001).

Table 2.3. 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-flavour
Spurious particulates	Unsafe, reduced quality
Enzymatic activity	Browning, consistency / flavour changes
Dissolved oxygen	Browning, nutrient and quality reduced
Metallic cations	Flavour / colour / nutrient losses, unsafe
Maillard reactants	Browning, quality loss
Colloidal instability	Sedimentation / precipitation / haze
Extended holding	Quality deterioration

2.3.1. Spoilage Microorganisms and Pathogens in Fruit Juices

Fruit juice is a suitable medium for microorganisms grown in low acidic conditions causing spoilage and pathogenic microorganisms that can cause food illnesses (Tournas, et al. 2006). Therefore, the spoilage and contamination of a fruit juice are the major concerns and microorganisms must be prevented in the juice by applying several prevention methods in the processing (Tournas, et al. 2006; Vantarakis, et al. 2011; Bates, et al. 2001; Ray, 2004).

Fruit juice i.e. a low acidic beverage can be spoiled by moulds, yeasts and aciduric bacteria such as *Lactobacillus, Leuconostoc* and *Acetobacter* spp. which lead to off-flavour, odor and color deterioration. The spoilage depends on the type of microorganism and variety of the juice (Ray, 2004; Tournas, et al. 2006). For example, moulds and *Acetobacter* can grow in a condition which requires presence of dissolved oxygen in the juice. Yeasts not only tend to cause oxidation by generating H_2O and CO_2, but also ferment the juice and produce alcohol and CO_2. *Lactobacillus and Leuconostoc* spp. are the sources of bad taste in the juice by transforming citric and malic acids into lactic and acetic acids. *Acetobacter spp.* produce acetic acid by utilizing alcohol affecting the taste. *Lactobacillus fermentum* and *Leuconostoc mesenteroides* turn carbohydrates of the fruit juice to lactate, ethanol, acetate, CO_2, diacetyl and acetoin. *Leu. mesenteroides* and *Lab. plantarum* cause formation of residuals by producing dextran and other exopolysaccharides (Ray, 2004).

Federal register [FD] (2001) announced *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* are responsible pathogenic microorganisms resulting from the food illnesses arising by consumption of contaminated fruit juice (Table 2.4). It was demonstrated that *E.coli* and *Salmonella* were the main responsible microorganisms for occurrence of outbreaks in the juices. As an addition to this, parasitic protozoa such as *Cryptosporidium parvum* has also been reported that it can survive in the unpasteurized fruit and vegetable juices (Pathanibul, et al. 2009; Gabriel and Nakano, 2009; Cook, et al. 1998). In the literature, it was indicated that the origin of contamination of fruit juices by pathogens was due to the consumption of unpasteurized and fresh juices (Bates, et al. 2001).

Table 2.4. Fruit juices – associated food poisoning outbreaks.
(Source: Bates, et al. 2001)

JUICE (YEAR)	INFECTIOUS AGENT	JUICE (YEAR)	INFECTIOUS AGENT
Sweet cider (1923)	*Salmonella typhi*	**Orange juice (1994)**	Gastroenteritis agent
Orange juice (1944)	*S. typhi*	**Orange juice (1995)**	*S. hartford, S. gaminera, S. rubislaw*
Orange juice (1962)	Hepatitis A	**Apple juice (1996)**	*E. coli* O157:H7
Orange juice (1966)	Gastroenteritis agent	**Apple juice (1996)**	*E. coli* O157:H7
Apple cider (1974)	*S. typhimurium*	**Apple juice (1996)**	*Cryptosporidium parvum*
Apple cider (1980)	*Enterotoxigenic E. coli*	**Apple cider (1997)**	*E. coli* O157
Orange juice (1989)	*S. typhi*	**Orange juice (1998)**	*Salmonella enterica*
Apple cider (1991)	*E.coli* O157:H7	**Orange juice (1999)**	*Salmonella muenchen*
Orange juice (1992)	*Enterotoxigenic E. coli*	**Orange juice (1999)**	*E. coli* O157:H7
Apple cider (1993)	*E. coli* O157:H7	**Apple cider (1999)**	*Salmonella enterica*
Apple cider (1993)	*Cryptosporidium spp.*	**Orange, grapefruit, lemonade (2000)**	*Salmonella*
Carrot juice (1993)	*C. Botulinum*	**Apple cider (1998)**	*E. coli* O157:H7
Watermelon juice (1993)	*Salmonella spp.*	**Mamey juice (1999)**	*Salmonella typhi*

Foodborne outbreak systems of CDC (The Centers for Disease Control and Prevention) recorded 21 outbreaks related to juice consumption in the United States between 1995 and 2005. Among these outbreaks, ten outbreaks were related to apple juice or cider consumption, eight of these were related to orange juice consumption and remaining three illnesses were connected with other kind of juices. 1.366 illnesses were reported during these 21 outbreaks. Out of ten, half of these outbreaks were due to *Salmonell*a and *E.coli* O157:H7, other two were caused by *Cryptosporidium* and one outbreak was related to Shiga toxin that was produced by *E. coli* O111 and *Cryptosporidium* (Vojdani, et al. 2008).

The United States Food and Drug Administration (USFDA) implemented Hazard Analysis and Critical Control Point (HACCP) programme in order to inhibit outbreaks caused by pathogens contaminating fruit juices (Pathanibul, et al. 2009; Gabriel and Nakano, 2009). In this programme, 5 log reduction in the number of the most resistant pathogen is targeted according to relevant regulation (21 CFR 110) (USFDA, 2001).

2.3.2. Enzymes Activities in Fruit Juices

Existence of enzymes sometimes provides desirable effect in the juice processing, but they are usually essential problems that eventuate the quality loss in the juice (Falguera, et al. 2011; Cemeroğlu, 2004). Polyphenol oxidase (PPO) (or cathechol oxidase), peroxidase (POD) and lipoxygenase (LOX) are the enzymes that are responsible for oxidation of the juice. Besides, pectinmethylesterase (PME) and polygalacturonase (PG) from pectinases result in cloudness and impact on viscosity of the juice (Schilling, et al. 2008; Timmermans, et al. 2011). Amylases may also present in the juice (Falguera, et al. 2011).

The most important enzyme from the juice processing point of view is polyphenoloxidase (PPO). Catechol oxidase is the most common form of PPO enzymes in the fruits. PPO utilise phenolic compounds in the juice and turn into the o-quinons. After a polymerization stage, brown pigments called melanins occur in the juice. These reactions that are known as "enzymatic browning reactions" in the food technology have adverse impact on the quality of the juice and should be inhibited during processing (Falguera, et al. 2011; Cemeroğlu, 2004). Peroxidises (POD) are also important enzymes in the processing of fruits and vegetables. They catalyse the oxidation reactions in presence of hydrogen peroxide conduce to off-flavour, color and nutritional quality loss. The inactivation of these enzymes are important in the thermal treatment of juices because they are the most resistant enzymes to the heat (Riener, 2008; Aguilo´-Aguayo, 2010; Cemeroğlu, 2004).

Although, Lipoxygenase (LOX) activity is not very common in the fruit juice,but in the case of present in large quantity, this enzyme not only supports the oxidation of polyunsaturated fatty acids, but also causes to co-oxidation of carotenoids and formation of volatile compounds such as aldehydes that can be significant in development of off-flavor (Aguilo´ -Aguayo, et al. 2010).

Pectinases are important enzymes in the clarification step of the juice processing. Pectins present in the juice have unfavourable effect because of their considerably viscous structure (Rai and De, 2009). Before clarification, pectinases such as Pectinmethylesterase (PME) and polygalacturanese (PG) hydrolyse pectins, pectin complexes with protein and help to remove them from the juice by aggregation (Cemeroğlu, 2004; Rai and De, 2009). PME catalyse partially de-esterification of pectin

by acting on C6 methyl ester groups of pectins (Wicker, et al. 2003). PME generates a substrate for PG providing depolymerization of pectin (Salvia-Trujillo, et al. 2011). The inactivation of these pectolytic enzymes is necessary in order to prohibit sedimented or cloudiness in juice (Falguera, 2011).

In order to have efficient clarification of the juice, amylases are also needed to be inactivated. Amylases break up starch in the fruit composition and have an effect on cloudiness similar to pectinases. When the inactivation of pectolytic and amylolytic enzymes are insufficient, the juice becomes unstable during storage (Cemeroğlu, 2004).

2.4. Fruit Juice Preservations

The main goal in the application of protection methods is based on elimination of spoilage and pathogenic microorganisms, and enzymes from the juice. Preservation methods applied to foods should have these following properties;

The method must:

- extend the shelf life of the food and inactivate the pathogen and spoilage microorganisms
- not affect the organoleptic and nutritional quality of the food
- not have residues after processing
- have low cost and be practical
- not have any negative effect on consumers (Raso and Barbosa-Canovas, 2003).

The most conventional preservation method is known as thermal processing (Aguilo-Aguayo, 2010; Rajashekhara, et al. 2000; Pathanibul, et al. 2009; Igual, et al. 2010; Valappil, et al. 2009). Other than heating, alternative non-thermal technologies such as antimicrobials, filtration, pulse electric field (PEF), high hydrostatic pressure (HHP), ultrasound, oscillating magnetic fields (OMF) and ultraviolet radiation (UV-C) are also used in the juice processing (Butz and Tauscher, 2002).

2.4.1. Thermal Pasteurization

2.4.1.1. Technology and Efficiency of the Thermal Pasteurization

Conventional thermal treatment is a widespread method used for pasteurization of the juice. (Valappil, et al. 2009; Aguilar-Rosas, et al. 2007; Tiwari, et. al., 2009).The objective of a thermal pasteurization or low-heat processing is to kill all vegetative pathogen microorganisms and approximately 90% of the relevant microorganisms (spoilage microorganisms etc.) such as yeasts, moulds, bacteria and viruses. Addition to killing of microorganisms, enzymes that are present naturally in the food are also inactivated by pasteurization. In order to obtain safe and high quality foods, thermal pasteurization is performed as continuous HTST (high temperature short time) and UHT (ultra high temperature), and aseptic packaging (Rivas, et al. 2006). Thermal pasteurization is applied between 100 ˚C and 60 ˚C for a couple of seconds in the fruit juice processing industry in case of juice pH is equal or less than 4.5 (Rivas, et al. 2006). Generally, in the pasteurization, juices are treated with the average heat as 90 ˚C and 1 min to provide long shelf life of the juice (Timmermans, 2011). For example, thermal pasteurization is implemented at 90-95 ˚C for 15-30 s in the processing of white grape juice, while this temperature and time are 96 C for 15-30 s and 90 ˚C for 60 s for the apple juice and orange juice, respectively. Grape juice is very sensitive to heat causing a cooking taste very quickly. Therefore, it is heated at lower temperature than other juices (Cemeroğlu, 2004).

According to FDA, 5 log reduction in the number of the most resistant microorganism is aimed in the pasteurization. FDA reported that pathogens such as *E. coli* O157:H7 and *Listeria monocytogenes* can be the main target microorganisms for pasteurization because they are very resistant to heat (USFDA, 2001).

Thermal pasteurization studies that were performed to inactivate of target pathogens and spoilage microorganisms in the juices were often reported in the literatures. According to the Gabriel and Nakano (2009), *E.coli* O157:H7 strain is the most heat resistant microorganism among the others and 4.43 min at 55 ˚C was necessary to pasteurize the clear apple juice. Walkling-Ribeiro et al. (2008) reported 8 log reduction in the number of *S. aureus (*SST 2.4) inoculated to apple juice reconstituted from concentrate subjected to pasteurization at 94 ˚C for 26 s. Elez-

Martinez, et al. (2005) inoculated orange juice with *Lactobacillus brevis* (CECT 216) and pasteurized at 90 ˚C for 1 min. They achieved 4.3 log reduction in the number of targeted microorganism. On the other hand, Wu et al. (2005) applied thermal pasteurization to inactivate natural microorganisms found in red and white grape juices. Inefficient inactivation was achieved in red and white grape juices because of mild heat treatment applied from 42 to 52 ˚C. Although approximately 2 log reductions of yeasts were obtained at the temperatures of 50 and 51 ˚C for red grape juice, these numbers scaled up to 3 log reductions at 52˚C. Considering white grape juice, 2 log reductions of yeasts were observed at 50˚C temperature (Wu, et al. 2005).

2.4.1.2. Impacts of Thermal Pasteurization on the Juice Quality Parameters

Significant microbial reductions and extended shelf life were reported by thermal pasteurization of the fruit juices.. However, sensory and nutritive characteristics of the juices are affected adversely and several problems such as permanent loss of flavour and taste, degradation of nutrients and undesirable browning reactions are generated by heating (Walkling-Ribeiro, et al. 2008; Elez- Martinez, et al. 2005; Garde-Cerdán et al. 2007; Charles-Rodriguez, et al. 2007).

Timmermans, et al. (2011) demonstrated the effect of mild heat pasteurization on the fresh squeezed orange juice during two months storage by controlling some of the quality parameters such as pH, brix, color, viscosity and cloudness. After pre-heating to 45 ˚C and pasterization at 72 ˚C for 20s, pH and brix were not different just after treatment and during storage when compared to untreated orange juice. Cloud stability and viscosity did not change because of inactivation of pectinmethylesterase (PME). Color parameters L*, a* and b* showed some variety in comparison to unpasteurized orange juice. Higher L* (lightness) and b* (yellowness) values and less a* (redness) value were observed after mild heat treatment indicating lighter, more yellow and less redness in juice.

Zhang et al. (2011) were specified differences of certain quality parameters on the basis of PME residual level of thermally processed and unprocessed watermelon juice. At the 60 ˚C and 60 min thermal treatment provided 50% inactivation of PME. After these treatment, less lighter, more red and yellow watermelon juice was obtained

when L*, a* and b* color values were compared. Total color differences (ΔE) were also 6.94. Browning degree of the juice was increased by rising of pasteurization time from 5 to 60 min at 60˚C. Dynamic viscosity was not very different from unpasteurized juice.

Charles-Rodriguez et al. (2007) investigated the effectiveness of heat treatment on fresh squeezed apple juice inoculated with E.coli O157:H7. They were used pH and color of the juice as quality parameters in comparing conventional HTST pasteurization and control juice. More than 5 log reductions were achieved. Higher pH value was detected in the HTST pasteurization. The reason for this could be explained by evaporation of organic acids of the juice. Additionally, at the higher pH value, the juice more tends to spoil. It was observed that L* value decreased, a* and b* values increased with the pasteurization indicating the browning formation due to high temperature effect.

Garde-Cerdan et al. (2007) studied the thermal pasteurization of grape juice. After processing at 90 ˚C for 60 s, necessary microbial reduction was achieved. Some physicochemical properties such as pH, total acidity and reduced sugar content of thermally pasteurized grape juice were measured and no significant differences were detected.

2.4.2. Alternative Non-Thermal Preservation Methods

The "non-thermal" expression is generally used for technologies that have nonhazardous dosages of temperature. Currently, consumers have required more healthy, nutritive and fresh like foods. Therefore, food manufacturers have concentrated on the application of non-thermal technologies to the foods in order to minimize several damages such as degradations of nutrients, off-color and off-flavour of the products caused by thermal pasteurization (Pereira and Vincete, 2010; Artes and Allende, 2005). These non-thermal technologies are not only increasing the quality of the foods, but also they create less effect to the environment and have less processing costs, save more energy during processing and provide to develope additional value in the foods when comparing with the conventional thermal pasteurization method (Pereira and Vincete, 2010).

The main objective of the non-thermal preservations is "as little as possible processed, as much as necessary" (Butz and Tauscher, 2002). The necessary

pasteurization norm for the fruit juices is 5 log reduction in the number of most resistant pathogen according to the FDA (USFDA, 2001). Non-thermal pasteurization technologies are making an effort to alternate thermal pasteurization providing an effective preservation that are able to inactivate microorganisms and enzymes without causing any change in the nutritional and sensorial quality (Butz and Tauscher, 2002). Table 2.5 showed the advantages and disadvantages of the "as little as processed" juice. These alternative non-thermal methods are antimicrobial agents, pulse electric field (PEF), high hydrostatic pressure (HHP), ultrasound and ultraviolet (UV-C) irradiations. Table 2.6 demonstrated these several novel non-thermal technologies, generally.

Table 2.5. Advantages and Disadvantages of Minimally Processed Juice.
(Source: Bates, et al. 2001)

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

Table 2.6. New non-thermal food processing methods.
(Source: Butz and Tausher, 2002)

Process	Description	Critical factors	Mechanism of inactivation	Status
UV light	UV radiant exposure, at least 400 J/m2	Transmissivity of the product, the geometry, the power, wavelength and arrangement of light source(s), the product flow profile	DNA mutations induced by DNA absorption of the UV light	Used for disinfection of water supplies and food contact surfaces
Ultrasound	Energy generated by sound waves of 20,000 Hz or more	The heterogeneous and protective nature of food (e.g. inclusion of particulates) severely curtails the singular use of ultrasound for preservation	Intracellular cavitation (micro-mechanical shocks that disrupt cellular structural and functional components up cell lysis)	Combination with e.g. heat, pressure has certain potential
Oscillating magnetic fields (OMF)	Subjecting food sealed in plastic bags to 1–100 OMF pulses (5–500 kHz, 0–50˚C, 25–100 ms)	Consistent results concerning the efficacy of this method are needed	Controversial results on effects of magnetic fields on microbial populations	Application at the moment not considered
Pulse electric fields (PEF)	High voltage pulses to foods between two electrodes (<1 s; 20–80 kV/cm; exponentially decaying, square wave, bipolar, or oscillatory pulses at ambient, sub-ambient, or above ambient temperature)	Electric field intensity, pulse width, treatment time, temperature, pulse wave shapes, type, concentration and growth stage of microorganism, pH, antimicrobials, conductivity and medium ionic strength	Most theories studied are electrical breakdown and electroporation	Different laboratory-and pilot-scale treatment chambers designed and used for foods, only two industrial-scale PEF systems available
High pressure processing (HPP)	Liquid/solid foods, with/without packaging (100–800 MPa, below 0˚C to >100 C, from a few seconds to over 20 min) Instantaneously and uniformly throughout a mass of food independent of size, shape and food composition	Pressure, time at pressure, temperature (including adiabatic heating), pH, composition	Denaturation of enzymes, proteins; breakdown of biological membranes; cellular mass transfer affected	In use since 1990 (Japan, USA, France, Spain) Current pressure processes include batch and semi-continuous systems

2.5. Ultraviolet Light (UV-C) Irradiation

2.5.1. Ultraviolet (UV) Light

Ultraviolet (UV) light covers the wavelength region from 100 to 400 nm in an electromagnetic spectrum (Barbosa-Canovas, et al. 2005; Kouchma, et al. 2009).

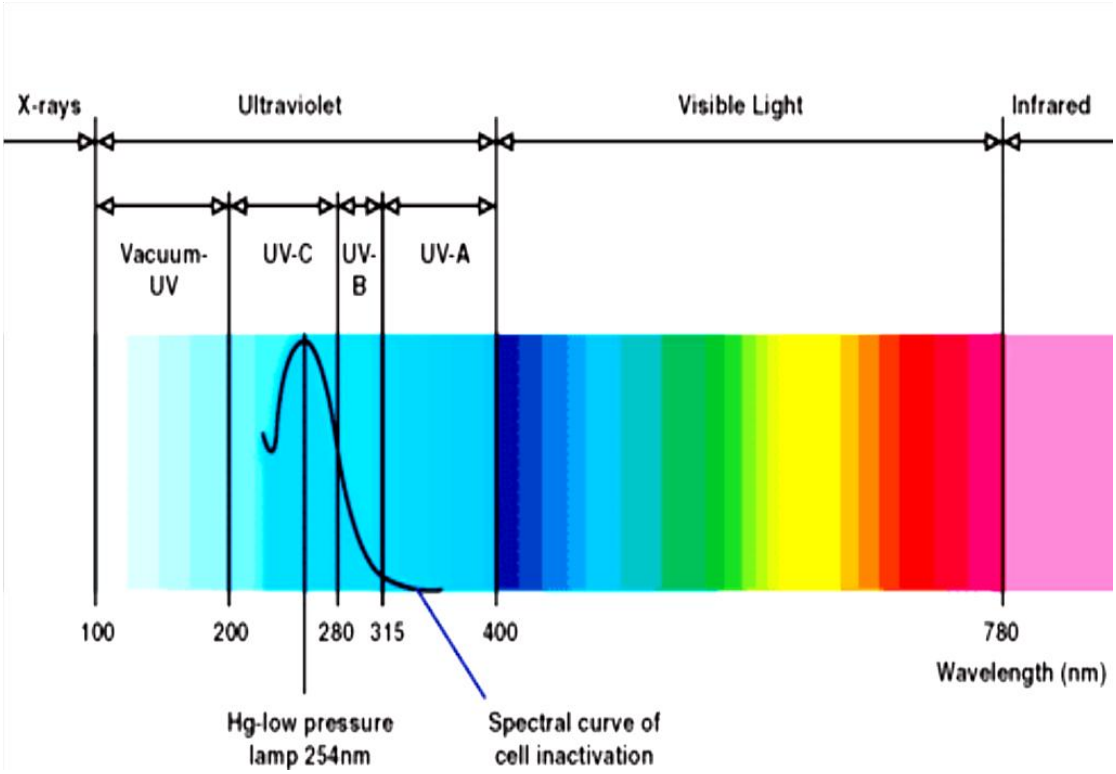


Figure 2.4. The electromagnetic spectrum.
(Source: Aquabest, 2007)

As it is shown in Figure 2.4, UV light region divided into four groups used in the processing technology. These are;

- UV-A (from 315 to 400 nm): long wave ultraviolet light that is responsible for damages named as tanning in the human skin,
- UV-B (from 280 to 315 nm): medium wave ultraviolet light that gives rise to burn of skin and skin cancer,

- UV-C (from 100 to 280 nm): short wave ultraviolet light known as germicidal spectrum, it causes destroying microorganisms such as bacteria, viruses, molds and yeasts,
- UV-V (from 100 to 200 nm): called as vacuum UV range that is absorbed by all materials, transmission of light can be achieved with only vacuum conditions (Koutchma, et al. 2009; Karel and Lund, 2003; Begum, et al. 2009).

UV-C is absorbed entirely within a few hundred meters in the atmosphere, because its absorption is very fast, so that it cannot be measured in the nature (Koutchma, et al. 2009; Falguera, 2011).

Ultraviolet light between 250 and 260 nm can inactivate most of the microorganisms and can be generally used in water disinfection (Begum, et al. 2009; Fonseca and Rushing, 2006). 254 nm is the most influential wavelength since the absorption of UV light by DNA of microorganisms at this wavelength is at the highest level (Koutchma, et al. 2009; Green, et al. 2003). Wavelength of UV and concentration of the absorbing material affect the absorption of the light and the absorbtion mechanism is well defined with Lambert-Beer law. (Franz, et al. 2009).

Three types of lamps are used to generate UV light. These are low pressure (LP), low pressure, high output (LPHO) and medium pressure (MP). The lamps compose of the inert gas and mercury. When the lamp starts to run, mercury evaporates and release the UV light in order to turn into its normal energy state. LP mercury lamp produces 253,7 nm monochromatic UV light, while MP generates higher intensity polychromatic UV light. UV inactivation technology mainly uses LP lamp because of low operation temperature and power requirements (Jonhson, et al. 2010).

2.5.2. UV-C Irradiation Mechanisms

Disinfection effect of UV light depends on absorption of the photons by DNA or RNA of the microorganisms causing lethal injury in the cells (Karel and Lund, 2003). Microorganisms absorb UV-C light and any change in the physical state of electrons leads to breaking bounds of DNA which is responsible for survivability and reproduction of the cells (Beltrán and Cánovas, 2005; Murakami, et al. 2006). Bond formation is generated between adjacent thymine molecules in the same DNA strain

because of pyrimidine bases (tymine and cytosine) which are very sensitive to UV light and tend to make a covalently tied dimmers (Figure 2.5). These dimmers block the reproductions of cells preventing formation of the correct replication of microorganisms (Beltrán and Cánovas, 2005; Koutchma, et al. 2009). The effect of UV light on the DNA strains of microorganisms is based on overall UV energy absorbed by the cells. On the other hand, some species can be turned to undamaged state called as "photoreactivation" after UV exposure (Karel and Lund, 2003).

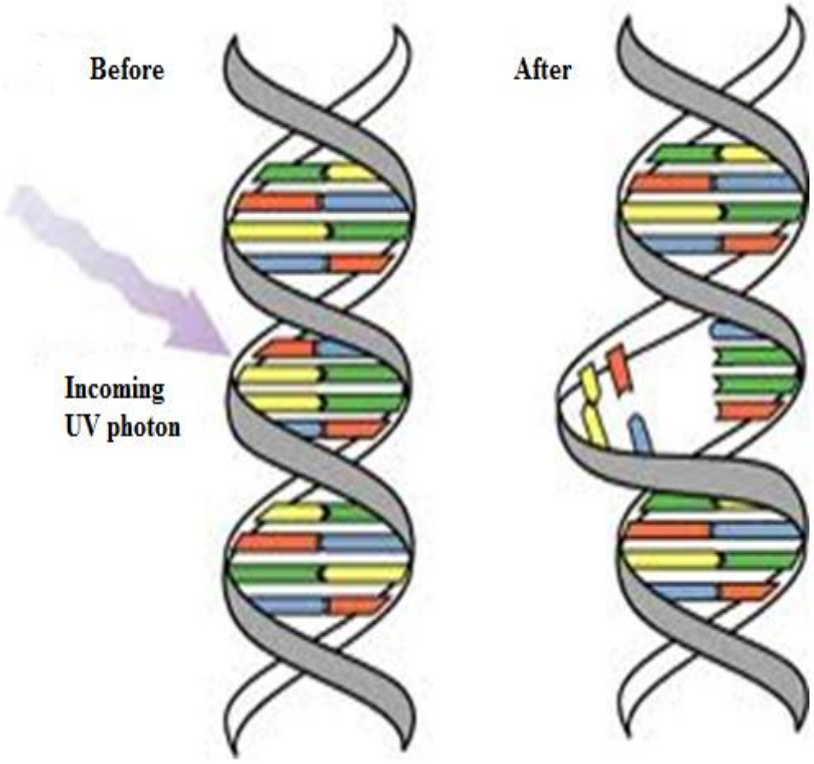


Figure 2.5. DNA structure before and after UV light absorption.
(Source: Koutchma, et al. 2009)

2.5.3. UV-C Disinfection of Microorganisms

Efficiency of the UV irradiation depends on the sensitivity of the different microorganisms to UV-C light. Several factors determine these sensitivities, these are; structure, thickness and composition of the cell-wall; UV absorbing proteins availability; varieties in the nucleic acid configuration (Koutchma, et al. 2009). Microorganisms can be categorised in terms of their resistance to UV-C light as; Gram negatives < Gram positives < yeasts < bacterial spores < moulds < viruses (Barbosa-Canovas, 2005).

Viruses are the most resistant microorganisms to UV-C light (Johnson, et al. 2010). Besides, it was reported that moulds such as *Sarcina lutea* and *Aspergillus niger* are very enduring cells as well (Cemeroğlu, et al. 2001). However, UV light can have more effect on protozoa such as *Cryptosporidium* and *Giardia* than other microorganisms (Johnson, et al. 2010; Barbosa-Canovas, et al. 2005).

Johnson et al. (2010) correlated the efficiency of UV inactivation with the size of microorganisms. The smallest cells are the most resistant to UV light due to the amount of UV absorbed per cell. Microorganisms larger than 1 μ have a less endurance because the absorption of UV-C irradiation by the cell is very high. However, yeasts and moulds are less sensitive to UV light, because they have less pyrimidine bases and have different cell membrane and thickness (Tran and Farid, 2004).

Table 2.7. UV dose requirement of some microorganisms for complete inactivation. (Source: Wyckomaruv, 2011)

Microorganisms	**UV dose (mWs/cm²)**
Salmonella enteritidis	7.6
Bacillus subtilis	11
Bacillus subtilis spores	22
Escherichia coli	6.6
Pseudomonas aeruginosa	10.5
S. typhimurium	15.2
Shigella paradysenteriae	3.2
Staphylococcus aureus	6.6
Saccharomyces cerevisiae	13.2
Baker's yeast	6.6
Brewer's yeast	8.8
Aspergillus niger	330
Influenza	6.6
Paramecium	200
Penicillium expansum	22

Inactivation levels of microorganisms are directly connected with UV dose applied to the cell (Koutchma, et al. 2009). Table 2.7 demonstrates UV dose requirements to inactivate 99.9% of some microorganisms. According to the table,

bacteria such as *E. coli*, *B. subtilis*, *S. enteritidis*, *S. aureus* are more sensitive to UV light than yeasts such as *S. cerevisiae* and molds such as *A. niger*.

At the certain UV intensity exposure, microorganisms generally exhibit a sigmodial shape logarithmic survival curve (Figure 2.6). This type of curve is composed of three different regions including an injury phase (shoulder region) that occurs in the nonlethal UV dosage, a linear section where the cells rapidly destroyed, and a tailing phase which may be formed in the case of a presence of very resistant strain of organisms or suspended solids (Karel and Lund, 2003; Barbosa Canovas, et al. 2005).

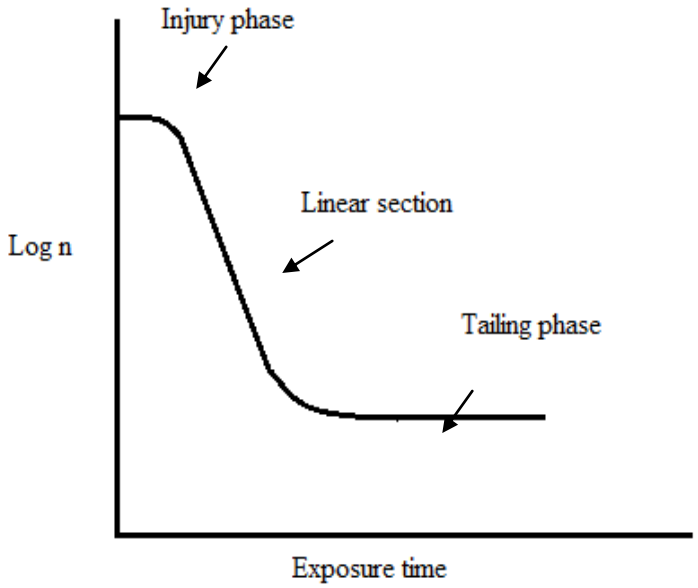


Figure 2.6. Typical survival curve of UV exposed microorganisms (log n: number of viable organisms) (Source: Karel and Lund, 2003).

2.5.4. Affecting Factors of UV-C Irradiations in Disinfection

For the UV-C irradiation of the foods, several factors should be taken into consideration in order to design an effective UV inactivation system. These factors can be summarized as;

- Variability of the species: UV sensivity changes for each type and species of microorganisms. For example, *Listeria monocytogenes* is more resistant bacteria to UV-C light than *E.coli* and *Salmonella* have been reported (Gabriel and Nakano, 2009),

- Number of microorganisms: an increase in the number of microorganisms requires more UV dosage for inactivation,
- Stage of microorganisms: the microorganisms at a logarithmic growth phase are more sensitive to UV light than the cells at stationary phase,
- UV-C irradiation conditions: suspended solids in the liquids lower the efficiency of the UV system due to preventing penetration of light,
- UV-C source: different UV lamps have different germicidal effect on microorganisms (Barbosa-Canovas, et al 2005).

Other than these factors, UV intensity, exposure time, flow rate of the liquid, surface area of the disinfection unit are other significant parameters needed to be taken into consideration for the successful UV inactivation process (Johnson, et al. 2010). In order to increase efficiency of the UV-C system, the flow regime and flow thicknesses are also important according to Koutchma et al. (2007).

2.5.5. UV Applications in Food Industry

Generally, UV-C irradiation applied for inactivation of the spoilage and pathogenic microorganisms and used in many areas in the food industry including disinfection of water, disinfection of air, surface disinfection of the foods and disinfection liquid foods and beverages (Koutchma, 2008; Koutchma, et al. 2009; Begum, et al. 2009).

Germicidal effect of the UV-C light is commonly used for water disinfection (Pereira and Vicente, 2010). It is a very effective, low costs technology in the drinking water industry because of having low costs and very low UV dosage to inactivate *Cryptosporidium* that is one of the important pathogens in water (Choi and Choi, 2010; Hijnen, et al. 2006). Water disinfection is also utilized in the brewing, soft drinks and cheese production (Tran and Farid, 2004). Other than drinking water, air disinfection is achieved by UV treatment in the related-food places such as the store rooms and kitchen hooms (Heraeus noblelight, 2011). UV inactivation can also be applied to protect from contamination of the surface of the foods such as ready to eat meats, baquettes, shell eggs, fresh cut fruits and whole fruits (Romo, 2004; Manzocco, et al. 2011; Chan, et al. 2009). Romo and Yousef (2005) were reported that inactivation of *S. Enterica* serotype Enteriditis in the shell eggs was ensured by UV irradiation.

Recently, UV disinfection is started to be used in the disinfection (or cold pasteurization) of liquid foods such as fruit juices, liquid egg products and beverages. These fluids have a low absorption of the UV because of presence of color compounds, organic matter, suspended solids, therefore UV efficiency is lower than other materials (Koutchma, 2008). But some of the fruit juices were successfully treated with UV-C light without causing significant changes in color, flavour and odor of the foods unlike in the thermal pasteurization (Keyser, et al. 2008; Murakami, et al. 2006). That's why, FDA approved the usage of UV irradiation as a non-thermal treatment method in the fruit juice processing instead of thermal pasteurization (US FDA, 2000).

2.5.6. Advantages & Disadvantages of UV-C Disinfection

Advantages of UV-C light disinfection are summarized as ;

- It is a non-thermal process. Off-flavor, off-odor, color and pH changes occur less in comparison to the thermal pasteurization (Barbosa-Canovas, 2005),
- It is environmentally friendly technology. Chemicals are not used in the UV-C process (Pereira and Vicente, 2010),
- It is introduced as a safe inactivation application for the foods by FDA. (USFDA, 2000),
- It can be set up easily and operating costs are low. This is an essential information for small-scale plants (Murakami, et al. 2006; Johnson, et al. 2010),
- It does not leave any residual after process (Artes and Allende, 2005),
- It can be used in combination with other technologies to increase effectiveness (Char, et al. 2010).

However, some disadvantages of the UV-C inactivation are listed as;

- It can injure the eye of a human whose not taking any precaution during UV treatment. Skin tanning or skin cancer may be generated in long exposure by UV-light (Pereira and Vicente, 2010)
- It can have adverse effect on the UV sensitive components such as vitamins, carotenes, tryptophan, unsaturated fatty acid residues in oils (Char, et al. 2010).

2.6. Studies of UV-C Irradiation in The Liquid Foods

There are numerous studies about the inactivation of spoilage and pathogenic microorganisms by UV-C light in liquid foods such as fruit juice, liquid egg products and beverages These are gathered mainly in two categories including the ones conducted by using a collimated beam-bench top UV apparatus (static UV systems) and a continuous flow UV systems where the liquid is passed through a UV reactor at a certain flow rate (dynamic UV systems) (Unluturk, et al. 2007 and 2010; Noci, et al. 2007; Murakami, et al. 2006; Gabriel and Nakano, 2009; Ngadi, et al. 2003; Walkling-Ribeiro, et al. 2008, Tran and Farid, 2004; Koutchma, et al. 2004 and 2006).

2.6.1. Bench Top UV Studies (Static UV Systems)

Ngadi et al. (2003) studied the inactivation of *E.coli* (O157:H7) in the apple juice by using collimated beam apparatus. They achieved 4.8 log reduction for 1 mm fluid depth at 6.5 mWmin/cm^2 UV dose in the apple juice. On the other hand, the reduction was only 3.5 log when the juice depth was 3.5 mm at the same UV dose. They realized that increasing the fluid depth resulted in less UV inactivation.

Similarly, Noci et al. (2008) investigated the effect of UV irradiation on the natural spoilage microorganisms in fresh squeezed apple juice. 2.2 log reduction was achieved exposing to UV light for 30 min using a 30 W UV lamp placed 30 cm distance from the juice. pH, brix and electrical conductivity of the juice were not affected. Color parameters (less L*, more a* and b*) indicated darker juice than a control sample. PPO and PME enzymes were not inactivated and total phenols and antioxidants in the juice were reduced by UV irradiation.

Gabriel and Nakano (2009) determined that *L. monocytogenes* (AS-1) cells were the most resistant bacteria to UV- irradiations than *E.coli*, *Salmonella* strains. 1.26 min decimal reduction (D value) was enough to pasteurize clear apple juice after exposure to a pair of 15 W UV-C lamp placed 55 cm away from the juice.

Unluturk et al. (2010) studied the inactivation effect of UV-C irradiation on different microorganisms inoculated into liquid egg white by using a collimated beam apparatus. They indicated that *E.coli* O157:H7 (NCTC12900, ATCC 8739) strain was more sensitive to UV irradiation than *E.coli* K-12 (ATCC 25253) and *Listeria innocua*

(NRRL B33314). They reported 1.403 log reduction after exposing liquid egg white samples spiked with this microorganism for 20 min at 26.44 mj/cm^2 UV dose.

2.6.2. Continuous Flow UV Studies

Continuous flow UV study was performed by Tran and Farid (2004). They measured aerobic, yeast and mould count in fresh squeezed orange juice after exposing to UV light using a thin film UV reactor. It was found that 87 and 119 mj/cm^2 UV dose were required for one decimal reduction of aerobic bacteria, and the yeast and mould count in the orange juice, respectively. Although PME enzyme was not inactivated, shelf life of the juice was extended to 5 days by 73.8 mJ/cm^2 UV dosage application. Vitamin C degradation was similar to thermal sterilization (17%) after exposing to 100 mj/cm^2 UV dosage. Color and pH of the juice were not changed significantly.

Koutchma et al. (2006) performed UV inactivation study using an apple cider inoculated with *E.coli K12* by means of Cidersure multiple-lamp laminar flow and Aquionics multiple-lamp turbulent flow UV reactor systems. Their paper demonstrated that different flow type on the UV inactivation of *E.coli K12* requires different UV decimal reduction dose. Aquionics UV reactor system required a UV dose ranging between 90 and 150 mj/cm^2 at 1250 ml/s flow rate for a decimal reduction. The same decimal reduction was achieved by applying a UV dose between 18.8 and 25.1 mj/cm^2 in thin film Cidersure UV reactor at flow rate of 56.8 ml/s. It was understood from this study that turbulent flow conditions was not very influential for high turbid fruit juice such as apple cider.

Keyser et al. (2008) used PureUV turbulent flow system for inactivation of microorganisms in the clear apple juice. After exposure of 234 mj/cm^2 UV dose (at a 66.6 ml/min flow rate), they achieved 3.5 log and 3 log reduction in the number of aerobic bacteria, and the yeast and mould count, respectively. Besides, approximately 5 log reduction was achieved for the inoculated *E.coli* strain in the apple juice.

CHAPTER 3

MATERIALS AND METHODS

3.1. Raw Materials

3.1.1. Pasteurized White Grape Juice

Commercial pasteurized white grape juice made from seedless Sultana grapes (Kavaklıdere, Ankara) were purchased from a local market.. Pasteurized grape juice was chosen because it does not have significant levels of indigenous background flora. Juice samples in the size of 200 ml bottles were stored at room temperature (20 ºC) until it was used.

3.1.2. Fresh Squeezed White Grape Juice

White grapes (seedless sultana) were purchased from a market place in İzmir. A household table top fruit juice extractor (Arçelik, Robolio, İstanbul) was used to mash the grapes without applying a washing step in order to supply an initial microbial load for spoilage of the juice. After the pressing step, white grape juice was strained twice by means of cheese cloths. In order to protect the color of juice, several amounts of L-ascorbic acid (200, 400 and 600 mg/kg) was added as an antioxidant (Codex stan. 82, 1981), 600 mg/kg L-ascorbic acid was selected eventually due to provide the best color preservation (Appendix B). The white grape juice was mixed in the shaker (Thermo Electron Corp., Ohio, USA) at 4˚C and 100 rpm, for 2-3 hours in order to precipitate potassium bitartrate. After removal of bitartrates from juice, it was packed in a plastic bottle and stored at the freezer (-18˚C).

3.2. Physical, Chemical, Optical and Rheological Measurements

Physical, chemical and optical properties of all the samples were measured before and after UV treatment.

3.2.1. pH

Determination of pH values of the commercial pasteurized and fresh squeezed white grape juice was carried out by using a bench top pH meter (HANNA Instruments, USA) at room temperature (20°C). Before measurements, pH meter was calibrated with buffer 1 (pH=7) and buffer 2 (pH=4), respectively.

3.2.2. Brix

Brix of the commercial and fresh squeezed white grape juice were determined by Mettler-Toledo RE40D Bench Top Refractometer (AEA Investors Inc., U.S.A) at 20° C calibrated with air and water.. Then, brix of white grape juice samples were measured within 30 seconds.

3.2.3. Titratable Acidity

Approximately 40 ml pasteurized and fresh squeezed white grape juice poured into a small flask and titrated with a standardized 0.1 N sodium hydroxide (NaOH) solution. Titration was stopped at pH 8.1 that is the phenolphthalein end point and the amount of NaOH used for titration was recorded. The percent of titratable acidity of the white grape juice samples (%) was determined according to the Equation 3.1. The acidity of the white grape juice is expresses in terms of tartaric acid because of tartaric acid is the dominant acid in the white grape juice.

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

V: the volume of 0.1 N NaOH used during titration (ml)

f: normality factor of NaOH from the table (0.9158 g)
E: miliequivalent weight of tartaric acid for 0.1 N NaOH (0.007505 g)
M: volume of the sample used (40 ml)

3.2.4. Absorbance

The absorbance of the pasteurized and fresh squeezed white grape juice samples was measured by means of a Carry 100 UV-Visible Spectrophotometer (Varian Inc., CA, USA) set at a wavelength of 254 nm. In order to determine absorbance coefficient, several dilutions were made in the range of 1:10, 1:25, 1:50, 1:100, 1:200, 1:500. The sample from each dilution was put into one square millimetre quartz cuvette and the absorbance was recorded by the spectrophotometer. The absorbance values and concentration of samples were drawn in a graph. The slope of the graph was the absorbance coefficient of the white grape juice.

3.2.5 Turbidity

Turbidity value of the samples were measured by a turbidimeter (Model 2100AN IS, HACH Company, USA). 45-50 ml of white grape juice samples poured into glass tubes of the device, tubes were cleaned with silicon oil and turbidity were measured twice for each tube. Results were expressed as Nephelometric Turbidity Unit (NTU).

3.2.6. Colour

Colour properties of the pasteurized and fresh squeezed white grape juice samples were determined by using Konica Minolta CR 400 Chromometer (Konica Inc, Japan). CIE L* (brightness-darkness), a* (redness-greenness), b* (yellowness-blueness) parameters were determined. Standard white sheet was used to calibrate the device before measurements. 10 ml from each white grape juice samples was poured into small-scale plastic petri dishes (50 mm diameter). White sheet was put on the petri dishes to protect measurement from the effects of other colors and results were recorded as averaged values obtained from three different places of the samples.

According to these values, total color change and browning index of a sample (ΔE) was calculated from the Equations 3.2 and 3.3:

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

$$\text{Browning index} = 100 * \frac{\frac{0{,}31\ (a* + 1{,}75\ L*)}{(5{,}645\ L* + a* - 3{,}012\ b*)}}{0{,}172} \quad (3.3)$$

3.2.7 Density

Portable type density meter (Kyoto Electronics DA, Japan) was used to measure the density of samples at a temperature range from 5°C up to pasteurization temperature (90°C). Therefore, densities of the commercial and fresh squeezed white grape juices were evaluated as a function of temperature.

3.2.8. Viscosity

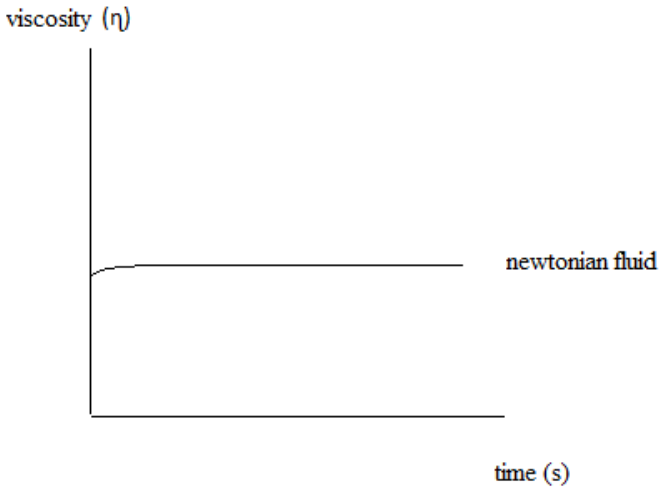


Figure 3.1. Time dependency of Newtonian fluids.

The flow behaviour of the fluids is important for selecting type of the process and equipment. (Chin, et al. 2009). Viscosity is defined as the ratio of shear stress

applied to the shear rate (Barker, 2008). This ratio is constant for Newtonian fluids such as water, milk and fruit juices (Nindo, et al. 2005). Figure 3.1. shows the time dependency of viscosity in the newtonian fluids. The measurement was based on a "controlled ramp test". This method helps to learn how the viscosity of the juice samples change as a function of shear rate or time.

Flow behaviour of the white grape juice was studied at a temperature range change between 5 ˚C and 90 ˚C (pasteurized temperature) increasing by five degree i.e. 5, 10, 20, 30, 40, 50, 60, 70, 80, 90˚C.

The viscosity of the pasteurized and fresh squeezed white grape juice was observed at each selected temperature by using concentric cylinder viscometer (Brookfield, DV II+Pro, Brookfield Engineering Lab. Inc., MA, USA) with a cylindrical spindle (UL-Y) at a constant rotational speed of 100 rpm (Figure 3.2). Shear stress and viscosity values were recorded by a software program of the viscometer (Rheocale32, Brookfield Eng. MA, USA) for every 10 seconds and total 40 data were collected per each temperature point. Viscosity versus time graph was generated to interpret flow behaviour of the juice samples.

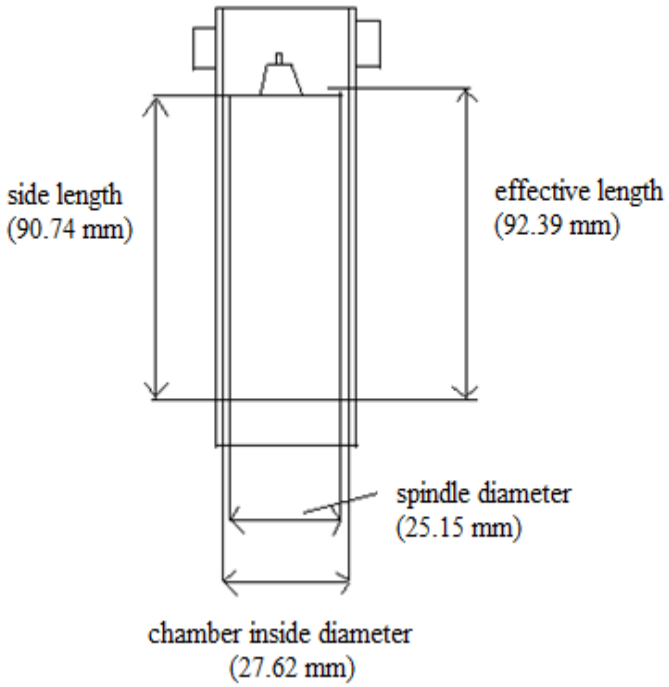


Figure 3.2. Dimensions of UL-Y Spindle.

3.2.9. Particle Size Analysis

The most important difference of the pasteurized and fresh squeezed white grape juice is that fresh squeezed white grape juice is a cloudy material having a turbid structure due to posessing insoluble particles. Particle size anaylsis of fresh squeezed white grape juice was carried out by a Mastersizer particle size analyser (Model: 2000, Malvern instruments Ltd., Malvern, UK). The equipment has hydro S small-volume general purpose dispersion unit and based on the laser diffraction technique. The instrument measures particle size of the sample within the range of 0.02 and 2000 μm with forward and back scattering of shortwave blue light. The light scattering method provides detection of the particle size distribution and average particle sizes of the insoluble materials found in the sample. The intervals of particle sizes of the samples depict the total volume of all particles in the distribution and the distribution of particle sizes is measured on the basis of volume of the particles (Betoret, et al. 2009).

3.3. Biodosimetric Studies

3.3.1. Target Microorganisms and Cultivation

3.3.1.1. Inoculation of *Saccharomyces cerevisiae* (Y 139) into Pasteurized White Grape Juice

Yeasts, lactic acid bacteria and moulds are the main spoilage microorganisms in the fruit juices **(**Tournas, et al. 2006). Therefore, in order to detect the effect of UV-C irradiation on the spoilage microorganisms in the clear pasteruized grape juice, *Saccharomyces ceresivisiae* was chosen as the target microorganism in this study. *Saccharomyces cerevisiae* (Y 139) was kindly provided by Dr. Handan Baysal from Food Engineering Department of Izmir Institute of Technology in Turkey. Stock cultures of *S. cerevisiae* (Y 139) were prepared using 20% glycerol and stored in cryovials at the freezer (-80˚C) until used. Cultivation of the *S. cerevisiae* strains was done by using Peptone-Yeast extract-Malt extract (PYM) Broth (prepared by manually according to literatures instructions: 10 g glycose, 5 g peptone, 3 g yeast extract, 3 g

malt extract per 1 L distilled water) and acidified to pH 4.5. Broth subculture was enriched after inoculation of 100 µl of *S. cerevisiae* from a stock culture into a flask containing 100 ml PYM broth and strains were incubated overnight by shaking 225 rpm in an orbital shaker (Thermo Electron Corp., Ohio, USA) at 30˚C. During incubation, the number of *S. cerevisiae* was determined every 2 hours by surface plating on PDA (Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) and optical density of the sample (i.e. absorbance) was measured in the Carry 100 UV-Visible Spectrophotometer (Varian Inc., CA, USA) at 600 nm wavelength. Logarithmic growth curve of *S. cerevisiae* was prepared by drawing the log survival numbers (CFU/ml) versus time (Figure 3.3). There was also a good correlation between logarithmic microbial count (log cfu/ml) and optical density (abs) (Figure 3.4.). *S. cerevisiae* (Y 139) reached to stationary phase at a concentration of ca. 8 log unit CFU/ml within 20 hours where the optical density was around 2.25. Figure 3.3. and 3.4 were used in the preparation of subcultures of *S. cerevisiae* (Y 139) for the inoculation of pasteurized grape juice samples. Inoculation of juice samples was carried out in the same way as explained in the previous step. After incubation, yeast cells were centrifuged at 3010 rpm for 15 minutes at 4 ˚C by a centrifuge (Rotina 380R, Hettich Centrifuge, UK) and cells were washed with 0.85% sodium chloride (NaCl) solution twice. The supernatant was removed and cells were inoculated into pasteurized white grape juice at a concentration of ca. 5-6 log unit CFU/ml. before subjecting to UV treatment. For enumeration, serial dilutions were prepared by using peptone phosphate buffered solution (Merck, Darmstadt, Germany). Cells were counted in Potato Dextrose Agar after incubation at 30˚C for 3-5 days.

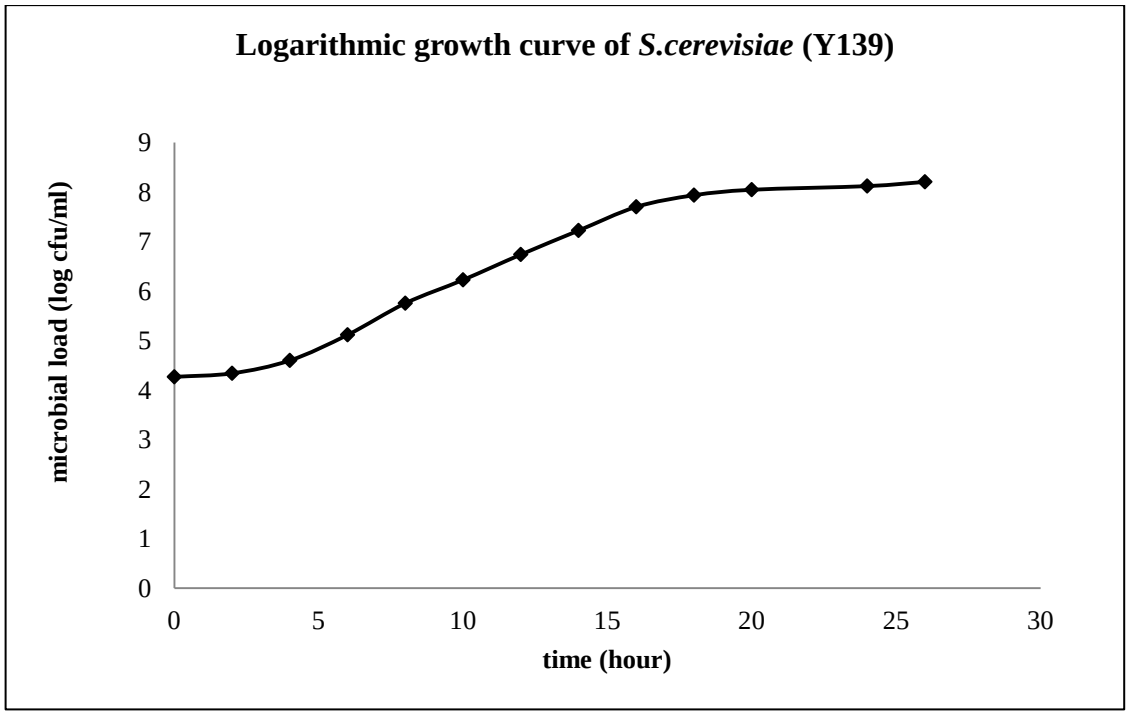


Figure 3.3. Logarithmic growth curve of the *S. cerevisiae* (Y139) strains in PYM broth (225 rpm, 30˚C).

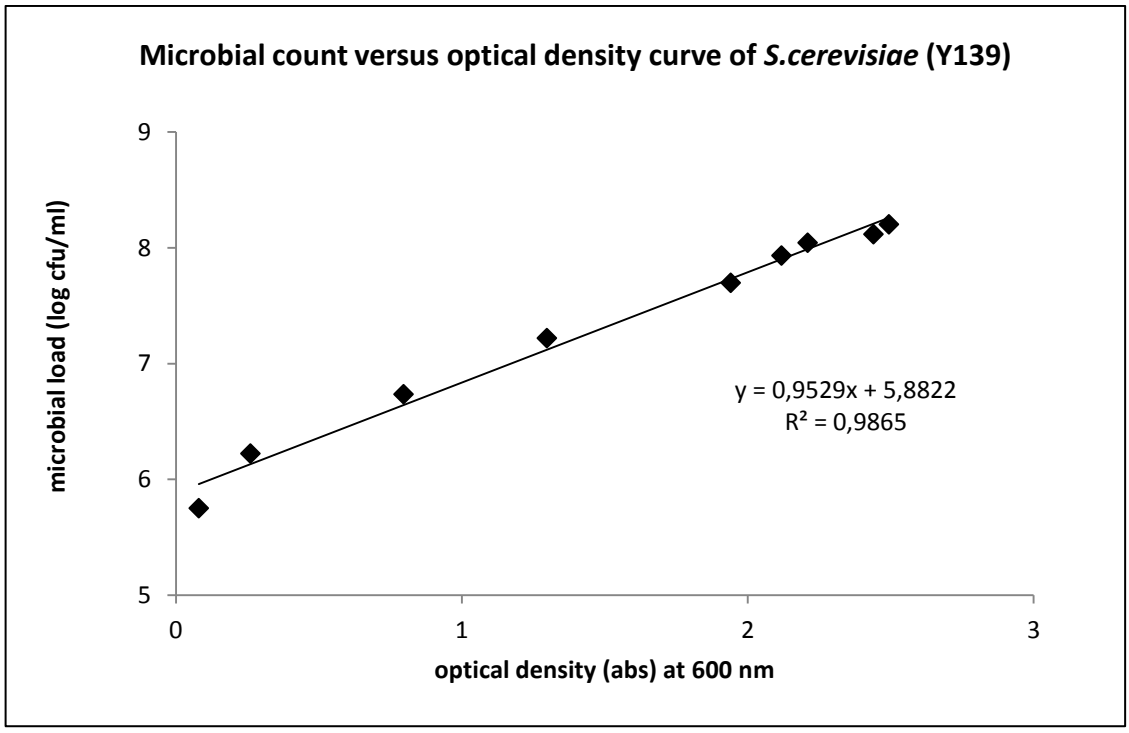


Figure 3.4. Logarithmic microbial count versus optical density curve of the *S. cerevisiae* (Y139) strains in PYM Broth.

3.3.1.2. Fermentation of Fresh Squeezed White Grape Juice

The white grape juice frozen in a 1 L plastic bottle was thawed and kept at room temperature (20°C) for one day to provide sedimentation of the suspended particles such as argols (potassium bitartrate or potassium hydrogen tartrate). After, the melted juice was stored at 4°C for one more day in order to increase the sedimentation effect (cold stabilization). Cold stabilization is the easy way to remove all tartrate crystals from the grape juice. Because tartaric acid and potassium exist in grapes bind together under chilly conditions, they form little potassium bitartrate crystals, which then settle to the bottom of the bottle. After melting and cold stabilization, the white grape juice collected on the upper part of the plastic bottle removed by a pipette (50 ml) without disturbing the sediment and naturally fermented (or spoiled) by stirring at 100 rpm and 30°C in the orbital shaker (Thermo Electron Corp., Ohio, USA) for 24 h. The juice was sampled every 3-4 hours and the microbial population was counted. A gram-stain was conducted on many samples and) the majority of cells were found to be yeasts and lactic acid bacteria.

Logarithmic microbial growth and optical density curve of the fresh squeezed white grape juice were generated (Figure 3.4 and 3.5), as in the case of pasteurized white grape juice (3.3.1.1). During fermentation, microbial population was counted on Tyriptic Soy Agar (TSA, Difco Laboratories, Detroit, Mich), Potato Dextrose Agar (PDA, Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) and De Man Rogosa and Sharp Agar (MRS, Merck, Darmstadt, Germany). Serial dilutions were prepared using peptone-phosphate-buffered solution (Merck, Darmstadt, Germany). The diluted samples were spread on TSA plates incubated at 37 °C for 18-24 hours to determine total aerobic count and PDA plates incubated at 25°C for 2-5 days for yeast count. Pour plate technique was used for MRS plates to count lactic acid bacteria. MRS plates were incubated at 30°C for 2 days. All the samples were plated in duplicate.

It is shown that total aerobic bacteria and lactic acid bacteria counts exhibit similar trend and numbers (Figure 3.4 and 3.5). Therefore we can assume that aerobic bacteria population are mostly contain lactic acid bacteria. Thus, it can be concluded that spoilage microorganisms in the white grape juice are mainly composed of yeasts and lactic acid bacteria.

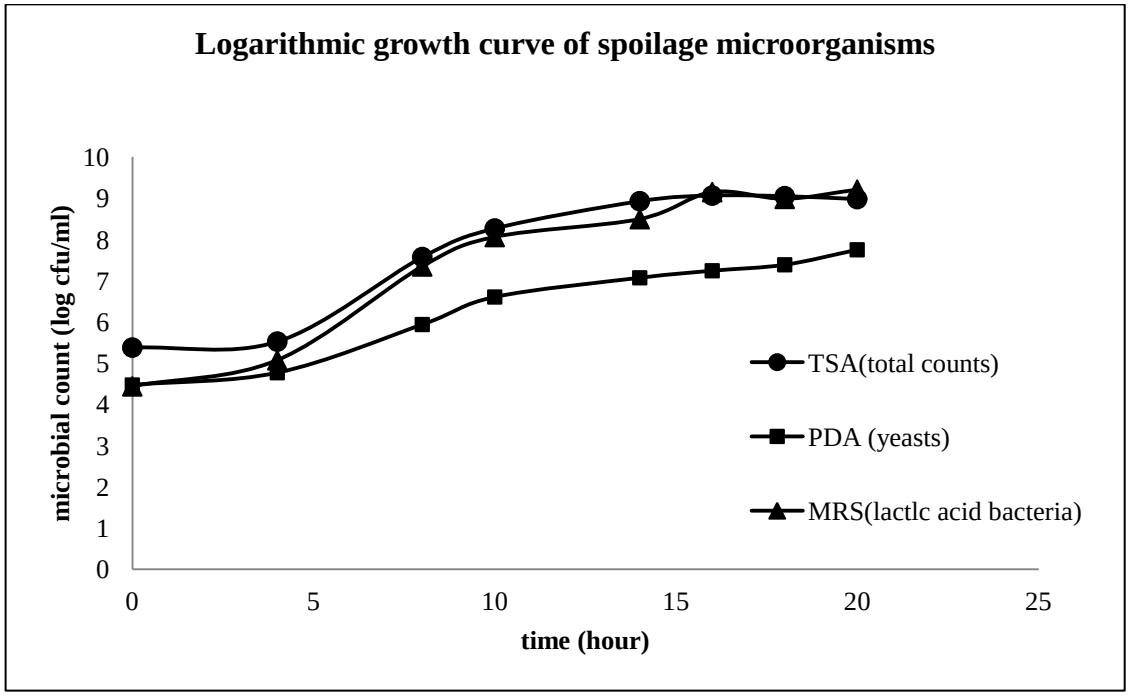


Figure 3.5. Logarithmic growth curve of spoilage microorganisms in the fresh squeezed white grape juice (100 rpm, 30˚C).

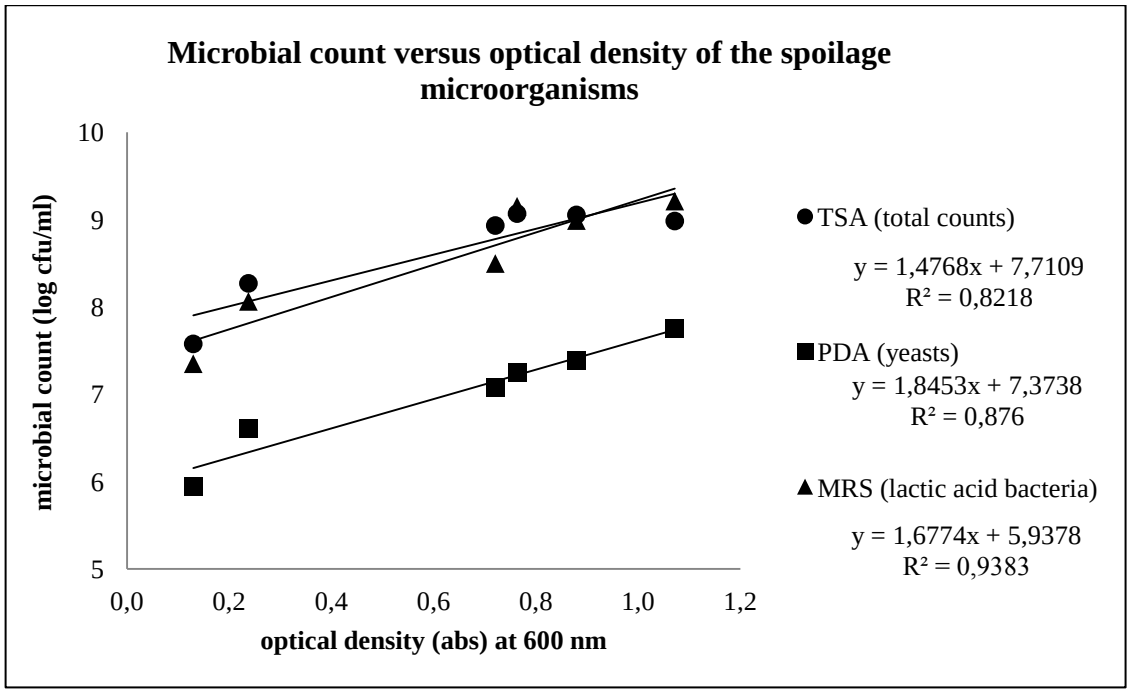


Figure 3.6. Logarithmic microbial count versus optical density curve of the spoilage microorganisms in the fresh squeezed white grape juice.

3.3.2. Bench Top UV Study

3.3.2.1. Collimated Beam Apparatus

The effect of UV-C irradiation on the inactivation of natural spoilage microorganisms in the fresh squeezed white grape juice and *S. cereviciae* in the pasteurized white grape juice was determined by means of a bench top UV system. Laboratory Scale Bench Top Collimated Beam UV apparatus was constructed based on descriptions given in Bolton et al. (2003) (Figure 3.7.). This equipment is made up of two identical low pressure mercury vapour UV lamps at 254 nm wavelength (UVP XX-15, UVP Inc., CA, USA) placed on the top of the apparatus. A shutter manually operated was positioned between UV lamps and samples to control of UV-C light exposure during experiments. UV light is passed through an opening (9 cm in diameter) which is in the size of a standard petri dish.and collimated through the sample placed just below the lamps by means of a black card board. The Bench top system has several trays located at different distance from the UV source to adjust UV dosage for the samples. A stirrer (IKA, Yellowline TTS 2, IKA® Werke GmbH & Co. KG, Germany) is positioned on a selected tray and provide mixing of the samples during exposure to UV light. Juice samples in petri dishes (50mm) were placed on the stirrer prior to measurement.

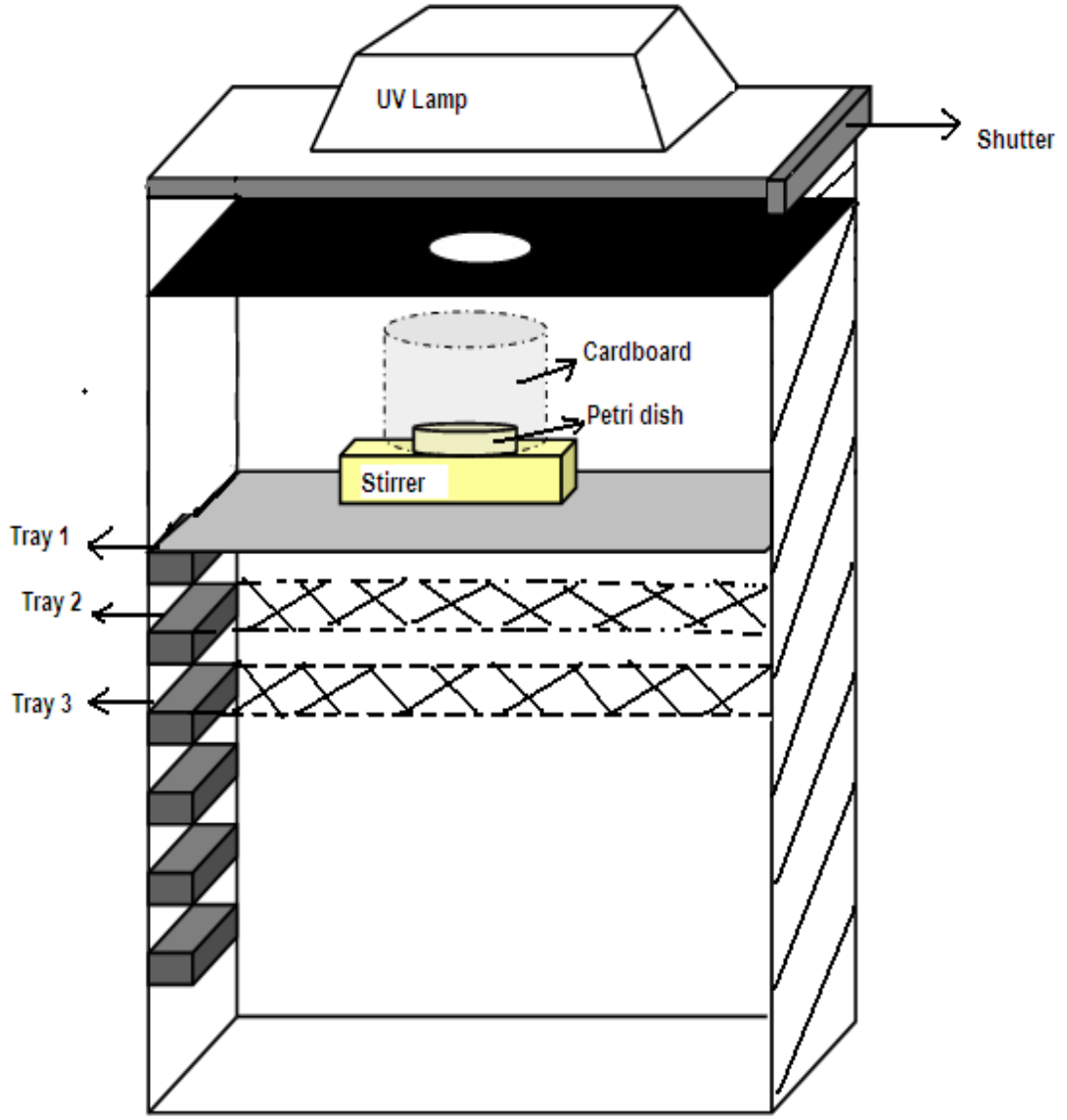


Figure 3.7. Laboratory Scale Closed Bench Top Collimated Beam Ultraviolet System. (Source: Atılgan, 2007)

Inside of the apparatus was painted to black colour and completely closed in order to minimize the scattering of UV-C light. All biodosimetric studies were performed in a closed system.. Before the experiment, UV lamps were switched on and hold approximately 30 minute to ensure complete activation of the lamps.. Intensity of the UV-C light on the sample surface was measured by using UVX Radiometer with UVX-25 sensor (UVX, UVP Inc. CA, USA) in mW/cm^2 before each experiment.

3.3.2.2. Inactivation Study by means of a Bench Top UV System

Pasteurized white grape juice inoculated with *S.cerevisiae* (Y139) strain at a concentration of 5.5-6 log cfu/ml and naturally spoiled fresh squeezed white grape juice samples containing 5.5-6 log cfu/ml yeast and 8 log cfu/ml of lactic acid bacteria were exposed to UV-C irradiation by means of a bench top collimated beam UV apparatus.

3 ml of the inoculated or fermented grape juice was poured into a 50 mm petrie dish to provide 0.153 cm sample depth. The petri dish was placed on the top of the trays (Tray 1), UV intensity on the surface of the pasteurized and fresh squeezed white grape juice samples was measured by means of a radiometer. Average UV intensity (average fluence rate or irradiation) passed through the juice samples was calculated according to Beer-Lambert law given in Equation 3.4.; (Unluturk, et al. 2008)

$$I_{avg} = I_0 * (1 - e^{-Ae*L}) / A_e * L \quad (3.4)$$

I_0 is the incident intensity of the sample surface (mW/cm^2), A_e is the absorbance coefficient of the samples (1/cm) and L is the path length of the samples (cm) in the Equation 3.4. UV dosage (d, mj/cm^2 or mWs/cm^2) applied to the samples was computed by multiplying average UV intensity of the samples (I_{avg}, mW/cm^2) with UV exposure time (t, s) according to the Equation 3.5.;

$$D = I_{avg} * t \quad (3.5)$$

After inoculation and fermentation step, the juice samples were exposed to UV-C light in the time interval between 0 and 21 minutes (0,3,6,9,12,15,16,18,21 min). At the end of each irradiation experiment, the survival number of microorganisms was determined by plating on (Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) and MRS Agar (Merck, Darmstadt, Germany).

The Equation 3.6 is used to calculate the survival ratio of microorganisms (S) after each UV irradiation. (Unluturk, et al. 2008)

$$S = \frac{N}{N_o} \quad (3.6)$$

Logarithmic inactivation rate of microorganisms was evaluated by Equation 3.7 (Chick, 1908). This equation is valid in the case of where there is a linear relation between survival ratio and UV dose or exposure time;

$$\text{Log}\ \left(\frac{N}{N_0}\right) = y - k * d \qquad (3.7)$$

No is the initial microbial count of the samples (cfu/ml), N is the microbial counts after UV exposure (cfu/ml), y is the intercept, k is the inactivation rate constant (cm^2/mJ) that calculated from the slope of the log N/No versus UV dose or exposure time curve and d if the decimal UV dosage (mJ/cm^2) or exposure time (min).

3.3.3. Continuous Flow UV Study

3.3.3.1. Continuous Flow UV Reactor

The main components of continuous flow UV reactor system used in this study are; (Figure 3.8, Table 3.1)

- A vertical UV reactor (Wedeco-Durco AG Water Technology, Germany) equipped with 6 low mercury UV lamps supplying an irradiation at 254 nm (Table 3.1) positioned outside a quartz tube
- Stainless steel tank (150 lt volume)
- 2M25 type stainless steel mono-pump (Inokstek Inc., Turkey) pumping liquid at a range between 100-1000 lt per hour adjusted with a frequency invertor
- Stainless steel piping system and equipments such as manual valves, elbows, non-aseptic sample valves
    - Stainless steel pipe (3.5 m length, 1" diameter)
    - Stainless steel pipe (1.1 m length, 2" diameter)
    - Quartz tube (1 m length, 2" diameter (Atılgan, 2007).

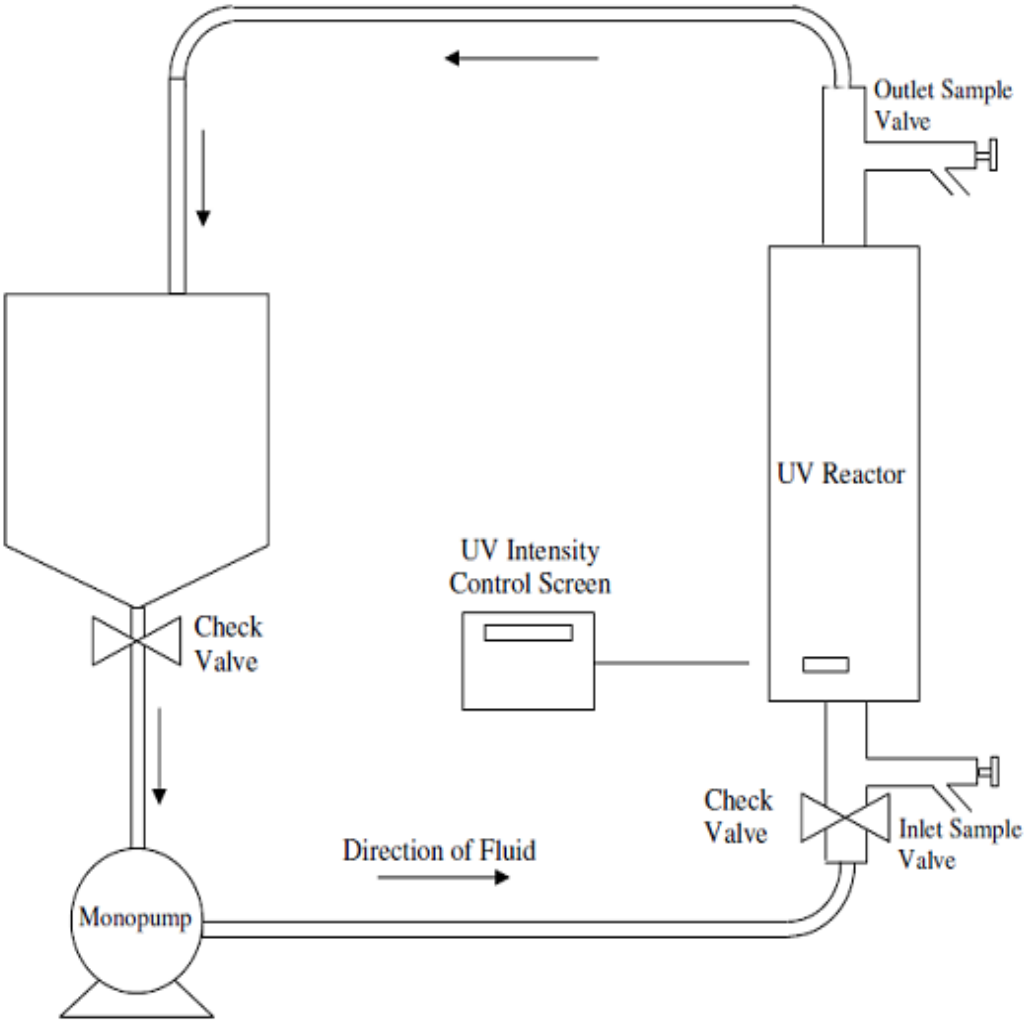


Figure 3.8. Continuous flow UV reactor system.
(Source: Atılgan, 2007)

Table 3.1. Options of Continuous Flow UV Reactor.
(Source: Wedeco-Durco AG Water Technology, Germany)

Reactor Type	E10
Reactor Connections: Male thread, stainless steel, 1.4571 (316 Ti)	R 2”
Width (mm)	240
Height (mm)	1090
Depth (mm)	245
Volume (lt) app.	4.5
Weight (kg) app.	31
Operating Pressure (bar)	10
Pressure Loss (bar) app.	0,01
Voltage (V/Hz)	230/50
Power Consumption (W/ V A)	230/500
Protection Class	IP 54
Lamp Type	NLR1579W
Lamp Power (W)	30
UV-C Output 254 nm (W)	12.5
Quantity	6
Expected Lamp Life Time (h)	8000

The system includes a vertical UV reactor that consists of six low pressure mercury UV lamps irradiating at 254 nm placed around a protective quartz tube. Reactor has a steel cover to provide a direct exposure of UV light to juice. The fermented juice in the amount of 9-10 lt hold in the stainless steel tank was pumped through the system at a defined flow rate regulated with a monopump and a frequency inventor attached to it. The fluid circulated in the system and the time necessary to complete one cycle was recorded from the moment after opening the check valves until the time when the first drop of juice had been seen on the tip of a returning pipe in the holding tank. Total time for one cycle of the system was calculated from Equation 3.8. Juice samples were taken from the inlet and outlet of the UV quartz tube after each cycle for microbiological analysis.

$$t_{total} = t_1 + t_2 + t_3 + t_4 \tag{3.8}$$

where t_1 is the time for the liquid to reach the UV reactor inlet, t_2 is the UV exposure time during the quartz tube, t_3 is the time necessary for liquid to return the tank after exiting UV tube and t_4 is the holding time in the tank before entering to the pipe. Total

residence UV time for eight cycles of continuous flow experiment is shown as t_{uv} in the Equation 3.9.

$$t_{uv} = 8 * t_2 \quad (3.9)$$

In addition to the total cycling time, volumetric flow rate (Q, ml/s) is measured during experiments to calculate Reynolds number (Re) used to predict the operating flow regime i.e. laminar or turbulent flow. For the fruit juice exhibiting Newtonian flow behavior, Re number is calculated from the Equation 3.10;

$$\text{Re} = \frac{D\ v\ \rho}{\eta} \quad \text{(for Newtonian fluids)} \quad (3.10)$$

where D is the diameter of the UV system (diameter of the quartz tube) (5.08 cm), v is the average flow velocity of the juice sample (m/s) , ρ is the density of the juice sample (kg/m^3) and η is the viscosity of the juice sample (Pa.s).

3.3.3.2. Inactivation Study by means of a Continuous Flow UV Reactor

The inactivation study of *S.cerevisiae* (Y139) strain in the pasteurized white grape juice and spoilage microorganisms in fresh squeezed white grape juice was carried out in a similar way as explained in section 3.3.2.2. It is very important to point out that the continuous system was disinfected by chemical agents and cleaned before each irradiation experiment.

S.cerevisiae cultivated in the 100 ml PYM broth stirred at 225 rpm and 30°C for 24 h was used to inoculate the pasteurized white grape juice. Inoculation procedure was explained in section 3.3.1.1. The total volume of inoculated juice was 8 lt and the final microbial population was around 5.9-5.95 log cfu/ml.

Fermentation of fresh squeezed white grape juice was explained in the section 3.3.1.2. After fermentation process, 100 ml of spoiled juice was used to inoculate approximately 9000 ml fresh squeezed white grape juice. Final microbial load of fresh squeezed white grape juice was composed of 5.5-5.7 log cfu/ml yeasts and 7.1-7.8 log cfu/ml lactic acid bacteria.

Pasteurized white grape juice with *S. cerevisiae* or fresh squeezed white grape juice with spoilage microorganisms poured into the stainless steel holding tank. Experiments were run at three frequency levels i.e. 20 Hz, 35 Hz and 50 Hz and volumetric flow rates at these levels were given in Table 3.2.

Table 3.2. Flow parameters of white grape juice samples at different frequency levels

White grape juice	**Frequency level (Hz)**	**volumetric flow rate (ml/s)**	**total cycling time (s) (t_{total})**	**Velocity (cm(s)**	**Re (Newtonian)**
Pasteurized	20	13.67	482	0.675	184.36
	35	25.27	403	1.248	340.83
	50	38	370	1.876	512.6
Freshly squeezed	20	12.9	480	0.636	163.86
	35	25.2	426	1.244	320.56
	50	34.75	377	1.715	442.76

UV penetration depth in the continuous flow UV system was low because the diameter of UV quartz tube was around 2" or 5.08 cm. Thus a long UV exposure time or numerous treatment cycle was necessary. In this study, eight treatment cycles were selected in order to inactivate microorganisms in the white grape juice samples. Microbial analysis was performed before and after each cycle by taking samples (approximately 50 ml) from the inlet and outlet of the UV quartz tube during each cycle. Samples were plated on PDA (Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) for determination of yeast count and MRS Agar (Merck, Darmstadt, Germany) for enumeration of lactic acid bacteria as explained in previous sections.

Additional to microbiological analysis, some amounts of samples were taken before and after each cycle to measure the physical, chemical and optical properties (pH, brix, density, titratable acidity, absorbance, turbidity, color). Therefore, the effect of UV-C irradiation on the quality parameters of the white grape juice samples were also investigated.

3.3.3.3. Cleaning of the Continuous Flow UV System

After UV inactivation study, the continuous flow UV reactor system was cleaned immediately by disinfectants and hot distilled water. 10 lt of 0.5 M NaOH solution prepared by dissolving of 200 mg sodium hydroxide (NaOH) (Panreac E-08110, Montcada I Reixac, Barcelona, Spain) in 10 lt boiled distilled water and pored into the stainless steel holding tank of the system. Solution was pumped through the system for approximately 15 minutes to remove any residues from the system completely. After removing of NaOH solution from the system, it was washed with 10-15 lt boiled distilled water for 15 minutes.

After that, the system was disinfected by 10 lt 2 % chloride solution (200 ppm chlorine) for 15 minutes, similarly. After disinfection, chlorine solution was washed by 15 lt boiled distilled water for about 15 minutes. UV-C lights were switch on during disinfection providing an extra microbial inactivation.

After sanitation of all the system, some amount of water sample was taken to control presence of microorganisms and residual chlorine in the system. Total microbial counts were analysed by spreading on to TSA (Difco Laboratories, Detroit, Mich) plates. Residual chlorine was controled according to related standard explained in the following section.

3.3.3.3.1. Control of Residual Chlorine After Cleaning step

Residual chlorine analysis was done according to 4500-CI B Iodometric Method I (American Public Health Association, 1995). The principle of the method is based on the release of free iodine of potassium iodide (KI) solution reacting with chlorine at pH 8 or less. Released iodine is titrated with a standard sodium thiosulphate solution ($Na_2S_2O_3$) in presence of indicator i.e. starch solution.

Firstly, standardized 0.1 N sodium thiosulphate solution ($Na_2S_2O_3$) was prepared. 25 g $Na_2S_2O_3$ was dissolved in 1 lt boiled distilled water and stored at least 2 weeks to allow oxidation of any bisulphate ion present. After storage, it was titrated with 0.1 N potassium dichromate ($K_2Cr_2O_7$) in presence of 0.1 N KI and conc. H_2SO_4. Therefore, normality of standardized potassium dichromate solution was prepared according to formula (Equation 3.11);

$$\text{Normality of } Na_2S_2O_3 = \frac{1}{ml\ \text{Na2S2O3 consumed}} \quad (3.11)$$

0.01 N or 0.025 N sodium thiosulphate solution that was needed in this analysis were diluted from the standardized solution. 100 ml water sample containing 1 g KI and 5 ml acetic acid, was titrated with standardized 0.01 N sodium thiosulphate solution ($Na_2S_2O_3$) until disappearing of yellow color resulted from free iodine occured after reaction with chlorine. After that, indicator starch solution (1 ml) was added and continued for the titration until removing blue color of the starch. Residual chlorine amount was calculated from the consumed sodium thiosulphate solution; (Equation 3.12)

$$\text{Total residual chlorine (mg as } Cl_2\text{ /ml)} = \frac{(\text{consumed Na2S2O3,ml}) * (\text{N}) * (35{,}45)}{\text{sample volume (ml)}} \quad (3.12)$$

where N is the normality of the sodium thiosulphate solution ($Na_2S_2O_3$).

3.3.4. Shelf Life Study

After UV irradiation of fresh squeezed white grape juice, shelf life study were performed in order to evaluate the effect of UV light on the juice spoilage. Fresh squeezed white grape juice (absorbance coefficient= 15.38 cm^{-1}) exposured to UV light by using Bench Top UV apparatus at a constant UV dose as 126 mJ/cm^2 (Io=0,78 mW/cm^2). Untreated juice and irradiated juice was stored at refrigerator temperature (4 ˚C) and counted on TSA (Darmstadt, Germany) for total plate count, PDA (Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) for yeasts and MRS Agar (Merck, Darmstadt, Germany) for lactic acid bacteria every 2-3 days during 13 days. In this study, the reference for microbiological spoilage was 5000 cfu/ml that was reported by Tran and Farid (2004).

CHAPTER 4

RESULTS AND DISCUSSION

This chapter covers the results that were obtained in this study. Physical, chemical, optical and rheological properties of pasteurized and freshly squeezed white grape juice were presented in the section 4.1. Results of biodosimetric study conducted for determination of UV dosage necessary to inactivate selected microorganisms in the pasteurized and freshly squeezed white grape juice samples by using Collimated Beam Bench Top UV equipment were given in the section 4.2. The results obatined for evaluation of efficiency of the continuous flow UV reactor system in the pasteurization of white grape juice samples were shown in the section 4.2. In addition to microbiological results, the effects of UVC light on some of the physical, chemical and optical properties of juice were studied before and after continuous flow UV inactivation treatments and the results were shown in the same section.

4.1. Physical, Chemical, Optical and Rheological Properties of White Grape Juice Samples

4.1.1. Physical and Chemical Properties

Some pyhsical and chemical properties (pH, density, brix, titratable acidity) of the pasteurized and fresh squeezed white grape juice were given in Table 4.1.

Sugar content (brix) values of pasteurized white grape juice and fresh squeezed white grape juice at room temperature (20˚C) were 17.24 ±0.035˚Brix (172.4 g/l) and 16.37 ±0.62 ˚Brix (or 163.7 g /l) from Table 4.1. These values were higher than 15 (%) which is the minimum range that needs to be provided for grape juices according to Codex Alimentarious Standards (World Health Organization and Food and Agriculture Organization of the United Nations, 1981). Garde-cerdan, et al. (2007) was reported that sugar content of grape juice ranged between 180 and 288 g/lt and our values were slightly below these values. The main reason for this might be due to the fact that grapes may show different characteristics from year to year in terms of location, soil or

seaosonal conditions (Bates, et al. 2001). On the other hand, Ekşi, et al. (1982) reported the average brix value of the grape juice as 17.03% and our findings are in good aggrement with that study.

Grape juice titratable acidity values were in the range of 300 and 555 mg/l in the in the study of Soyar et al. (2003) which is in a good correlation with values (364 mg/l) obtained for the pasteurized white grape juice used in this study. However, titratable acidity of the fresh squeezed white grape juice (0.194 ±0.03 mg/100ml) was less than pasteurized white grape juice (0.380 ±0.007 mg/100ml). The main reason of low pH (3.44 ±0.01) was that citric acid addition during processing of the commercial pasteurized white grape juice because citric acid in the pasteurized juice has higher degree of dissociation than ascorbic acid in the fresh juice. Besides, fresh squeezed white grape juice process was obtained only by pressing and detartarization or clarification step was not employed. The juice stored in a refrigerator temperature ((5 °C) to precipitate tartaric acid in the juice as argols (potassium hydrogen tartartares) and this might be the reason of having lower titradable acidity values. Similarly, lower titradable acidity caused higher pH values as 4.35 ±0.05 in the fresh squeezed white grape juice than pasteurized juice given as 3.44 ±0.01 (Table 4.1).

Density values were not affected by the processing step and found very close between for both pasteurized and fresh squeezed white grape juice samples in this study. Results also aggreed with Hakgüder (2009) findings. Ekşi, et al. (1982) indicated that average denstiy value was 1.073 g/cm^3 at room temperature (20˚C) for grape juice showing similarity with this study.

Table 4.1. Physical and Chemical Properties of White Grape Juice Samples.

White Grape Juice	**pH**	**Density (g/cm^3)**	**Brix (%)**	**Titradable acidity(%)**
Pasteurized	3.44 ±0.01	1.068 ±0.002	17.24 ±0.035	0.380 ±0.007
Fresh squeezed (after freezing)	4.35 ±0.05	1.065 ±0.001	16.37 ±0.62	0.194 ±0.03

4.1.2. Optical Properties

Table 4.2. depicts optical properties such as absorbance coefficient, turbidity, CIE color parameters (L*, a*, b*) and total color differences. of pasteurized and freshly squeezed white grape juice. It was noticed that there were some differences between two white grape juice samples.

Table 4.2. Optical properties of White Grape Juice Samples.

White Grape Juice	Absorbance coef. (^{1}/cm)	Turbidity (NTU)	Color				
			L*	a*	b*	ΔE	BI
Pasteurized	5.49 ±0.26	0.87 ±0.005	51.63 ±0.4	1.29 ±0.02	1.82 ± 0.24	51.68 ±0.39	57.50 ±0.15
Fresh squeezed (after freezing)	14.10 ±0.96	99 ±17	52.69 ±0.62	0.348 ±0.65	3.044 ±1.86	52.81 ± 0.69	57.80 ±0.82

Absorbance coefficients of the pasteurized and fresh squeezed white grape juice samples calculated from the slope of absorbance values. They were measured in the spectrometer at 254 nm and shown in Figure 4.1 and Figure 4.2. They were measured as 5.49±0.26 and 14.10±0.96 ^{1}/cm for pasteurized and fresh squeezed white grape juice, respectively. According to Beer-Lambert Law (Equation 3.4), UV light intensity and penetrating ability decrease with increase absorbance of the liquid (Murakami, et al. 2006). Therefore, results suggested that fresh squeezed white grape juice had more UV light requirement in order to inactivate microorganisms.

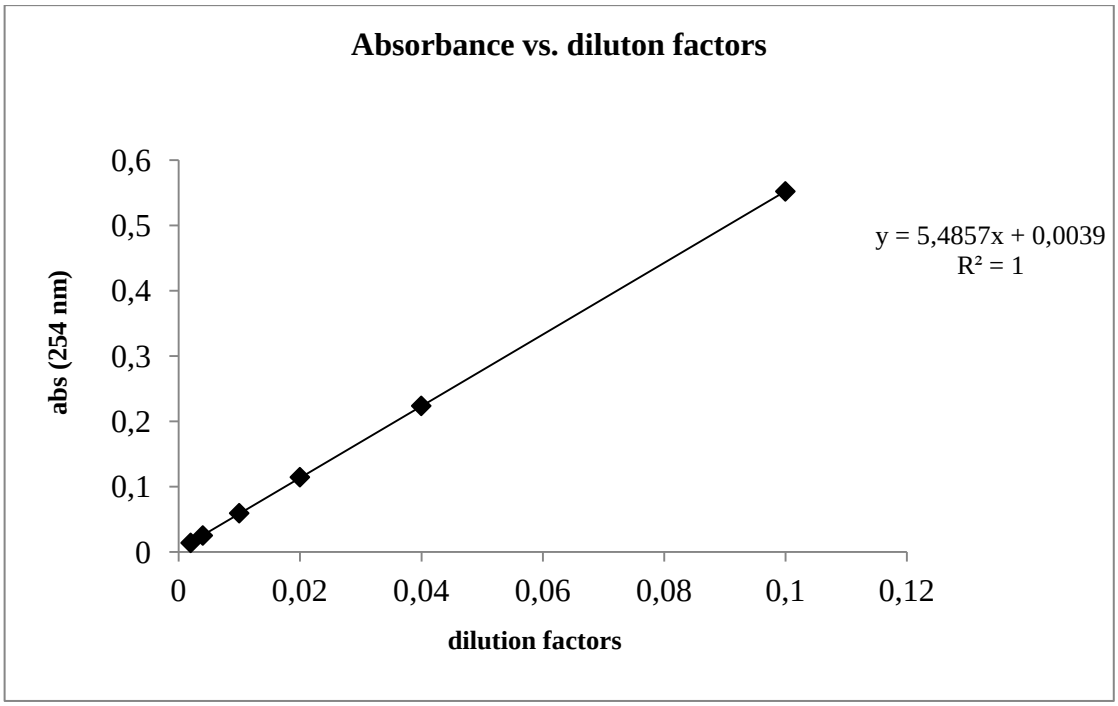


Figure 4.1. Absorbance Versus Dilution Factors Graph of Pasteurized White Grape Juice.

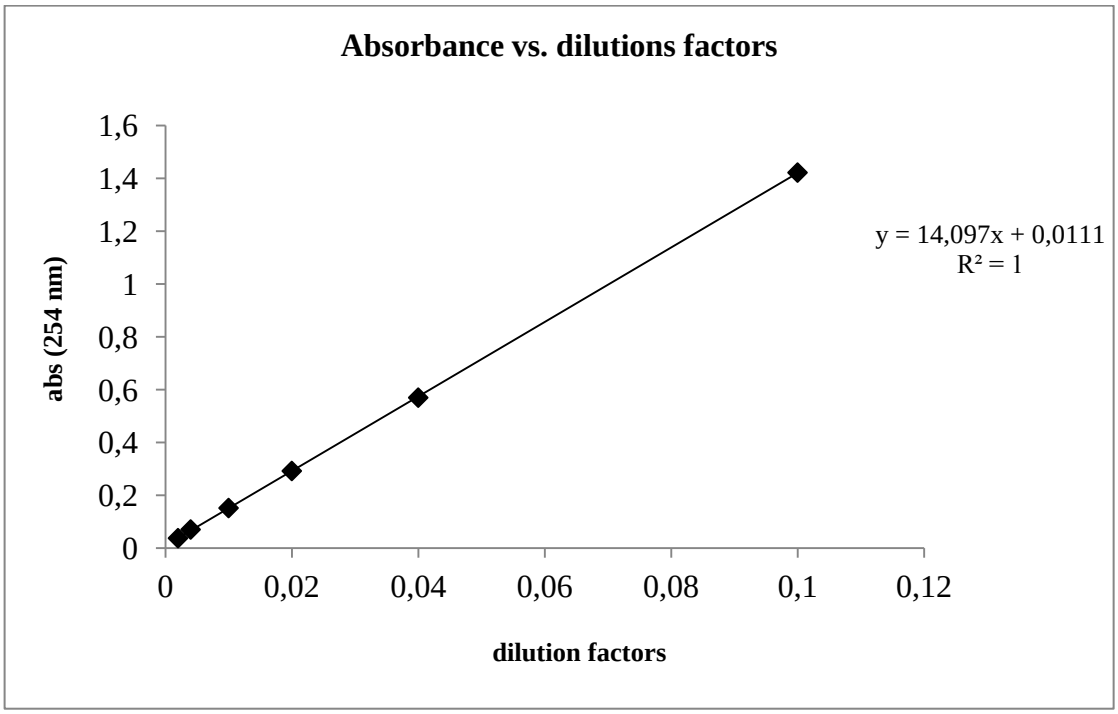


Figure 4.2. Absorbance Versus Dilution Factors Graph for Fresh squeezed White Grape Juice (before freezing).

Turbidity of the samples were changed greatly because of suspended particles. Although fresh squeezed white grape juice had many suspended solids in its composition and higher turbidity value as 99±17 NTU, pasteurized white grape juice was processed with a clarification step where suspended particles were removed. This step was resulted in less turbid juice as 0.87±0.005 NTU. But in the freshly squezed juice suspended particles mostly stay in the medium and cause tailing effect by inhibiting penetration of the UV-C light to the juice and consequently microorganisms.

The color parameters of juice samples were dissimilar although they were made of same kind of fruits. L* values of the juices indicated that fresh squeezed white grape juice (L*: 52.69±0.62) has a ligher color than pasteurized one L*: 51.63 ±0.4). The reason could be attributed to the heat treatment applied to white grape juice during pasteurization process. Pasteurization of the fruit juice causes browning that is represented as less L* value of the juice (Tiwari, et al. 2008). Similarly, b* value of the pasteurized white grape juice as 1.82±0.24 was less than fresh squeezed juice measured as 3.044±1.86. Therefore, fresh squeezed white grape juice implies to more yellowness related to caretonoid content in the juice composition. Pasteurized white grape juice had less carotenoid pigment comparing to fresh squeezed white grape juice due to carotenoid degradation caused by pasteurization process (Fratianni, et al. 2010). Also, suspended particles cause more yellowness in the fresh squeezed white grape juice due to the color of grape skin residuals. Parameter a* that is responsible for redness of the juice was higher in the pasteurized white grape juice. Literatures were reported higher a* values have also an effect on the browning of the fruit juice due to melanoidin that was occurred after non-enzymatic browning of the juice (Ibarz, et al. 2005). However, total color differences (ΔE) were different between two juice samples and browning index (BI) show that the fresh squeezed white grape juice was more brown color (57.80±0.82) than pasteurized juice (57.50±0.15). The reason may be that continuing enzymatic activity in the fresh juice cause enzymatic browning due to the lack of pasteurization step.

4.1.3. Rheological Properties

4.1.3.1. Density of the White Grape Juice Samples

Density values of the white grape juice samples were measured at a temperature range from 5˚C (the refrigerator temperature) to 90˚C (pasteurization temperature). In order to evaluate density value as a function of temperature measurements were carried out at each 10˚C of temperature increment by using Portable type density meter (Kyoto Electronics DA, Japan). Thus, sample density before UV irradiation experiments was calculated from a correlation derived from the temperature-density curves. This value was used in prediction of process parameters (Re, flow rate etc.) for continuous flow UV reactor.

Density results of pasteurized and fresh squeezed white grape juice at different temperatures were given in Figure 4.3 and 4.4. According to the tables, density values were decreased with increasing temperature. Third degrees polynomial models were applied to data in order to estimate density values of the white grape juice samples as a function of temperature. It was understood that there was a good correlation between density and temperature with r^2 values of 0.9954 for pasteurized white grape juice and 0.9903 for fresh squeezed white grape juice.

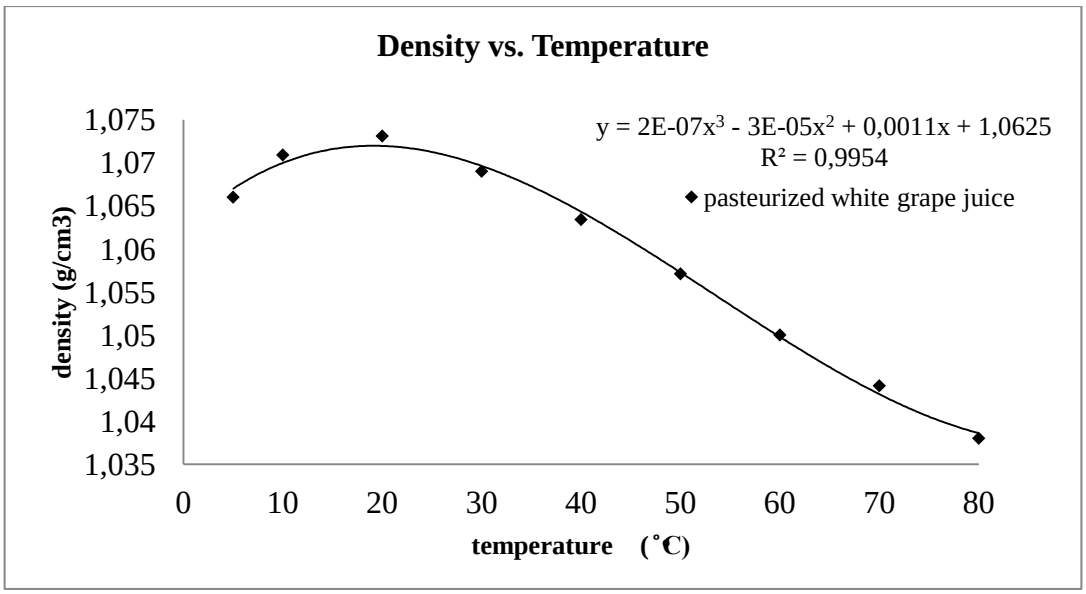


Figure 4.3. Density values versus temperature curve of pasteurized white grape juice.

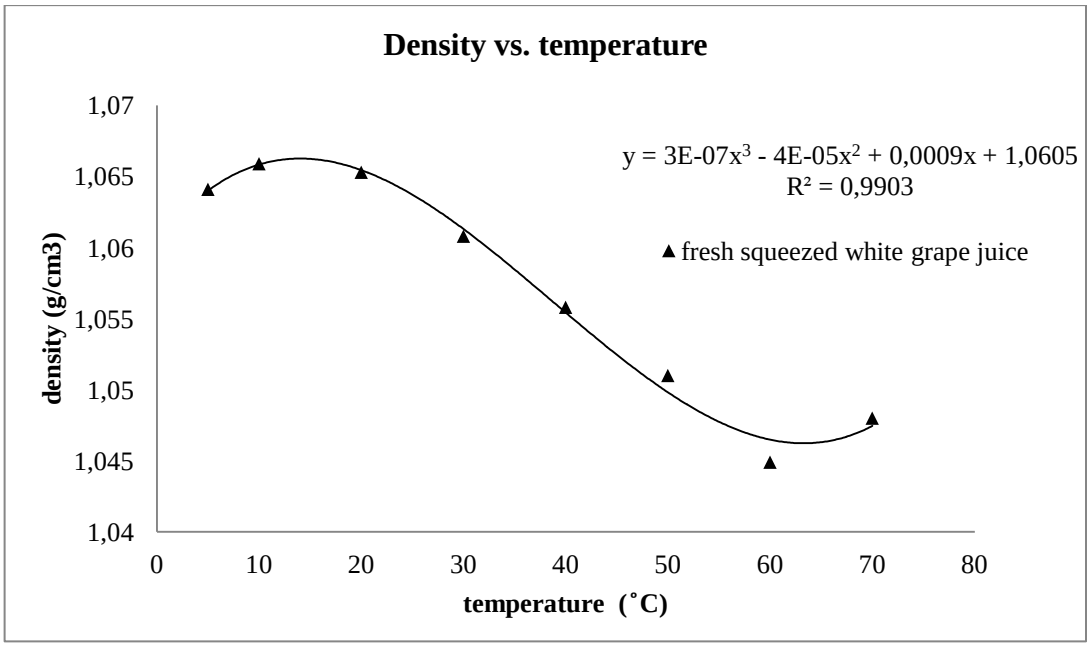


Figure 4.4. Density values versus temperature curve of fresh squeezed white grape juice.

4.1.3.2. Flow Behaviour of the White Grape Juice Samples

4.1.3.2.1. Time dependency

Fruit juices are usually newtonian fluids where the shear rate increases linearly by increasing shear stress and viscosity values are not affected by the time that is known as time-independed fluids. Viscosity stays constant by changing time (Nindo, et al. 2005). Therefore, shear rate that was studied in this study was selected as 100 rpm and time dependency of viscosity of the white grape juice samples were interpretted from the viscosity versus time graphs (Figure 4.5 and Figure 4.6).

Apparent viscosity of the pasteurized and fresh squeezed white grape juice samples were measured by a concentric cyclinder viscometer using a UL-Y cylindirical spindle at different temperature ranging from 5˚C to 90˚C. Viscosity graphs as a function of time were generated from 50 data that were obtained from the measurements recorded at every 10 seconds and viscosity behaviour of the samples was determined within total 500 seconds in this study.

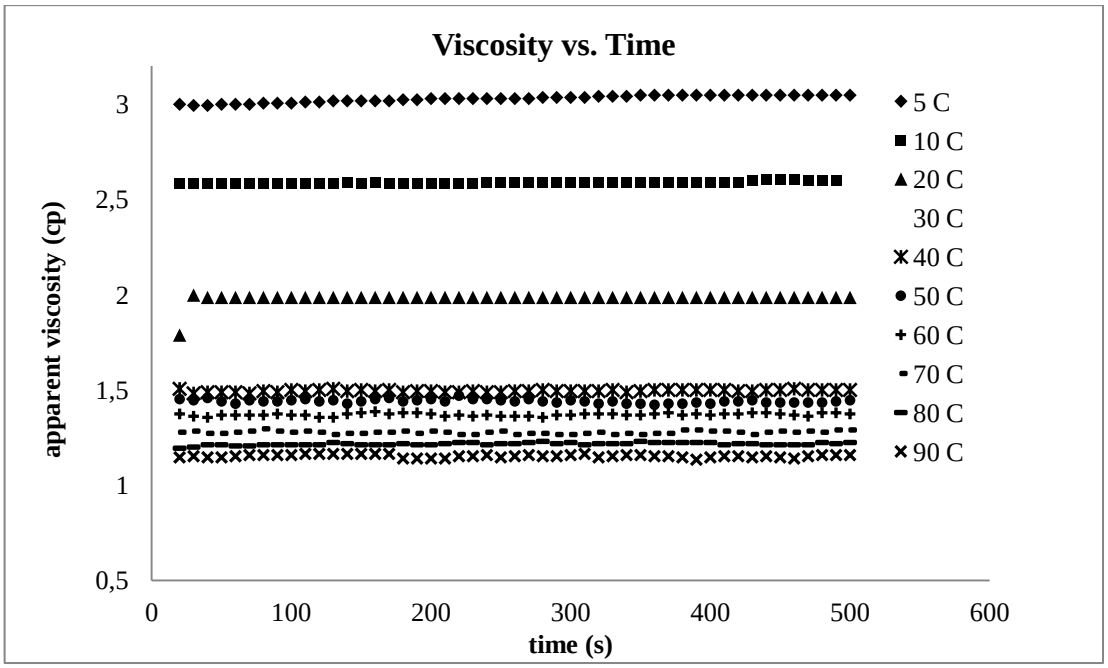


Figure 4.5. Time dependency of pasteurized white grape juice at a constant shear rate (100 rpm) and different temperatures.

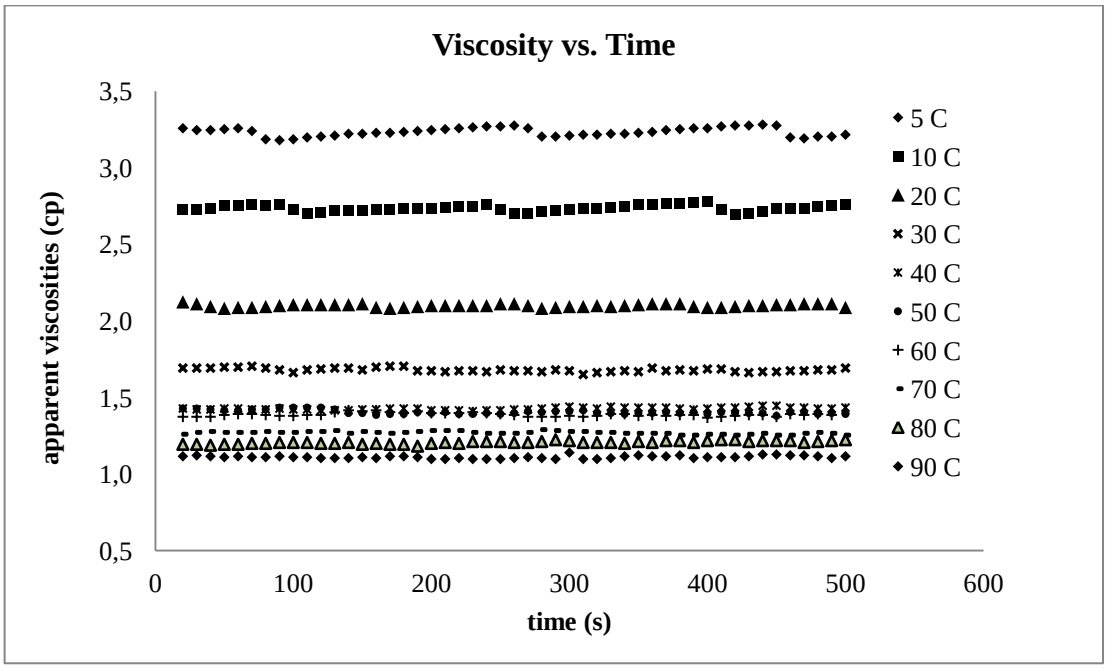


Figure 4.6. Time dependency of fresh squeezed white grape juice at a constant shear rate (100 rpm) and different temperature.

Figure 4.5 and 4.6 demanstrated that the apparant viscosity of pasteurized and fresh squeezed white grape juice samples were not changed by inreasing time showing

newtonian fluid behaviour at each temperature. The fluctuation in some of the data was attributed to the high soluble solid and suspended content of the fresh squeezed white grape juice (Zuritz, et al. 2005).

4.1.3.2.2. Temperature Dependency

Apparent viscosity was measured as a function of temperature for pasteurized and fresh squeezed white grape juice samples.

Fruit juices are newtonian fluids and only affected by temperature change. According to the Figure 4.7 and 4.8, results suggested that viscosity values of the both white grape juice samples decreased with increasing temperature. Polynomial models with third degree were applied to estimate viscosity values. There was a high relationship between viscosity and temperature of the juice samples according to the polynomial equations for pasteurized and fresh squeezed white grape juice with r^2 values of 0.9928 and 0.9975, respectively.

Viscosity values of fruit juices decrease by increasing temperature (Chin et al., 2009). According to their work, total soluble solids (˚Brix) in the fruit juices had an effect on viscosity increase. When comparing to the viscosity values of this study in the Figure 4.7 and 4.8, although brix value of fresh squeezed white grape juice was lower than pasteurized white grape juice, its viscosity was higher than pasteurized one at the same temperature because of the presence of suspended particles in its composition. Zuritz et al. (2009) also measured the viscosity of grape juice and found that soluble solids had a great impact on viscosity of the juice.

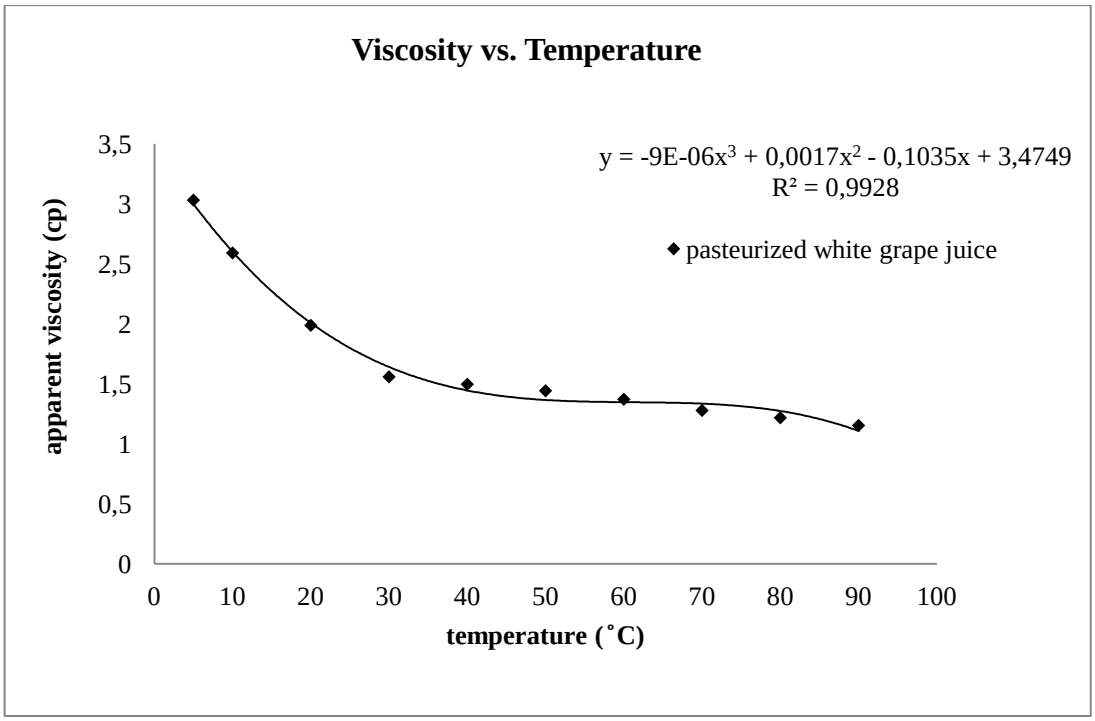


Figure 4.7. Temperature dependency of pasteurized white grape juice at a constant shear rate (100 rpm).

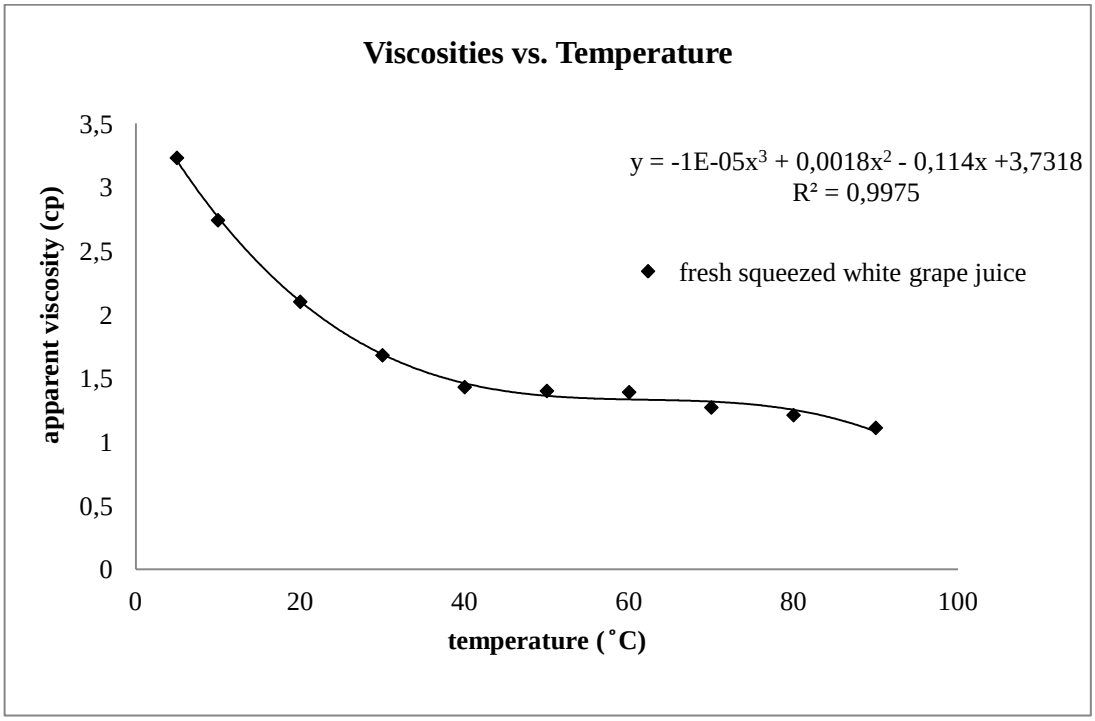


Figure 4.8. Temperature dependency of fresh squeezed white grape juice at a constant shear rate (100 rpm).

4.1.3.3. Particle Size Analysis of Fresh Squeezed White Grape Juice

Other than pasteurized white grape juice, fresh squeezed white grape juice have suspended particles in its composition. Particle size distrubution is important factor in evaluating of the efficiency of the UV irradiation and designing of the UV system, properly. Therefore, size of particles in the fresh squeezed white grape juice were analysed in Hydro S 2000 MasterSizer Particle Size Analyser. The sizes of suspended particles found to be changed between 0.2 and 2000 μm (Fig. 4.9).

Figure 4.9 showed general particle diameters (μm) of fresh squeezed white grape juice based on the volume % of the juice. Particle size was explicated in terms of the volume-weighted mean diameter (D[4,3]) calculated from Equation 4.1 and diameter at maximum peak of the graph (D_{peak}).

$$(\mathrm{D}[4,3]) = \sum_i n_i d_i^3 \,/\, \sum_i n_i d_i^4 \qquad (4.1)$$

ni symbolises the number of particles of diameter (di) (Betoret et al., 2009). According to the Figure 4.9, the maximum peak of particle size distributon in fresh squeezed white grape juice was 5.83 μm (D_{peak}) indicated that most existing particles in the total volume of the juice had a diameter of 5.83 μm. Additionaly, mean diameter over the volume distribution (D[4,3]) was detected as 32.549 μm from the Figure 4.9. Particle diamaters were distrubuted in a range between 0.24 and 316.228 μm in the distribution. Three main particle size distrubution were obtained. The first distribution was ranged between 0,240 and 0,631 μm; second and third one were changing between 0.631 and 69.183 μm and 69.183 and 316.228 μm, respectively. The first particle size distribution (small particles) was due to tartrates which is responsible from the cloudness of the juice. The third distribution (big particles) was attributed to large grape skins which could not be removed by filtration.

Particle size distribution (Span) of the fresh squeezed white grape juice also were calculated from the formula;

$$\text{Span} = \frac{\mathrm{d}(0.9) - \mathrm{d}(0.1)}{\mathrm{d}(0.5)} \qquad (4.2)$$

where d(0.1), d(0.5) and d(0.9) are the particle diameters determined at 10th, 50th and 90th percentile of undersized particle distribution curve at Figure 4.10. Span of the particle size distribution was found as 24.139 in this study.

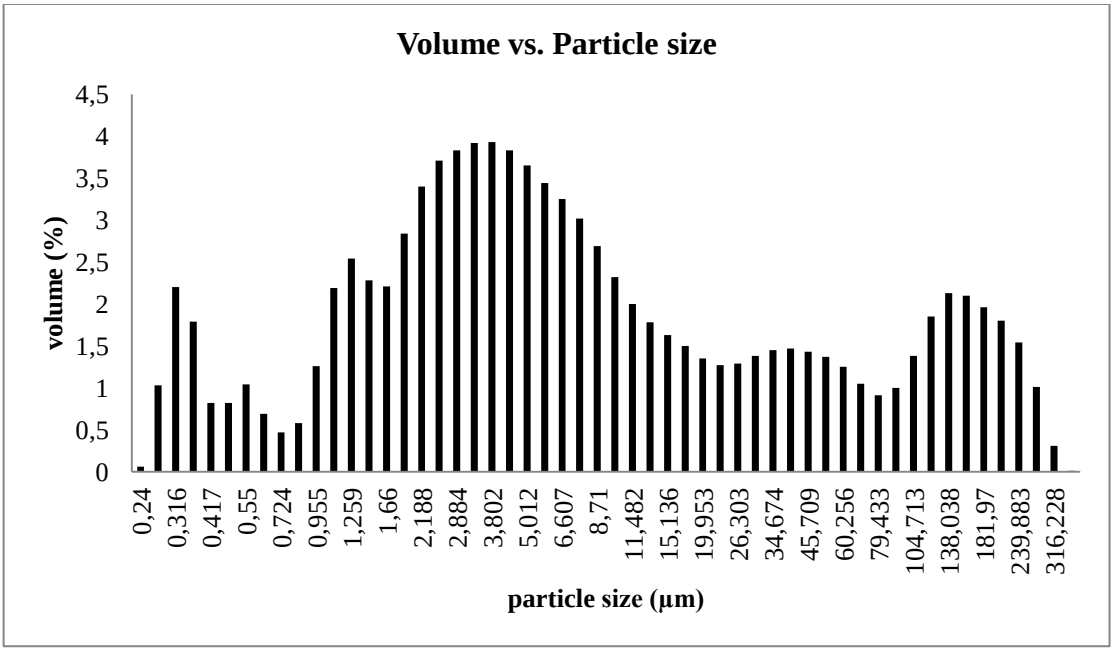


Figure 4.9. Particle size distrubution of fresh squeezed white grape juice.

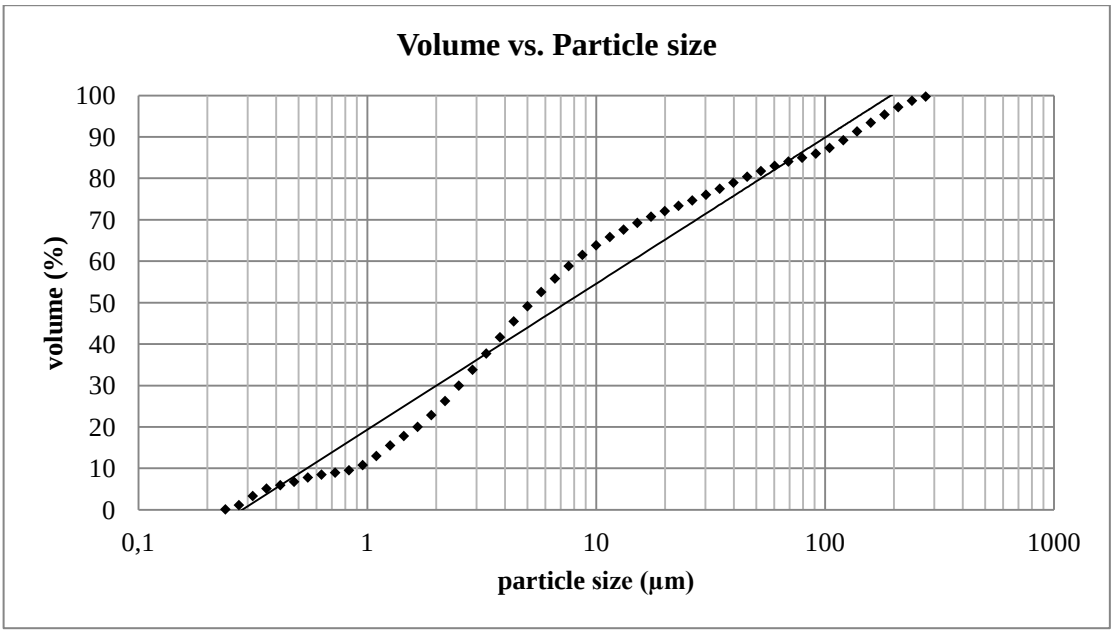


Figure 4.10. Particle size distribution in all the volume of the fresh squeezed white grape juice.

4.2. Biodosimetric Results

4.2.1. Bench Top Biodosimetric Results for White Grape Juice Samples

4.2.1.1. Pasteurized White Grape Juice

Before the Bench Top UV experiments, pasteurized white grape juice samples were tested to contol background flora of the juices. According to the total microbial count results on TSA, samples had no microorganisms in its composition. After microbial testing, *S.cerevisiae* strains in the numbers of 10^8 cfu/ml were inoculated to the pasteurized white grape juice. Final inoculation amount in the juice was adjusted to 5.50 ±0.107 log cfu/ml and 7.33 ±0.013 log cfu/ml for UV inactivation study. Therefore, the effect of two different initial microbial load on UV irradiation process were studied.

Table 4.3. Log reductions of pasteurized white grape juice at different initial microbial load (5.50±0.107 and 7.33±0.013 log cfu/ml) with two different intensities as Io=0.91 mW/cm^2 (I_{avg}= 0.636 mW/cm^2) and Io=0.36 mW/cm^2 (I_{avg}=0.252 mW/cm^2).

		UV intensity (mW/cm2)	**Time (min)**	**UV dosage (mJ/cm2)**	**Log reduction (log No/N)**
Initial microbial load (log cfu/ml)	**5.50±0.107**	0.91	3	114,48	4,34 ±0,3
		0.91	6	228.96	5.50 ±0.03
		0.36	3	45.36	3.33 ±0.2
		0.36	6	90.72	4.33 ±0.2
		0.36	9	136.08	5.43 ±0.01
	7.33±0.013	0.91	3	114.48	4.16 ±0.01
		0.91	6	228.96	7.32 ±0.009
		0.36	3	45.36	2.79 ±0.02
		0.36	6	90.72	3.97 ±0.01
		0.36	9	136.08	5.04 ±0.03
		0.36	12	181.44	7.33 ±0.03

Table 4.3 showed logarithmic reductions for applied UV dosages that was calculated from the exposure time (minutes) and incident UV-C light intensity

(mW/cm^2) measured on the surface of juice placed in a petri dish. Incident UV intensity is directly related to the distance between the sample surface and UV source. Two main intensity levels (0.91 and 0.36 mW/cm^2) were used to inactivate of *S. cerevisiae* in the pasteurized white grape juice having different initial load. Logarithmic reductions could be seen in Figures 4.11, 4.13 as a function of UV dosages and Figure 4.12 and 4.14 demonstrates the logarithmic reductions with applied exposure time, respectively.

The biodosimetric results showed that 228.96 mJ/cm^2 UV dose provided at the high intensity level (Io=0.91 mW/cm^2) was enough to inactivate all *S.cerevisiae* regardless of the initial load (Table 4.3). The survival curves were almost linear in these graphs. However, when the low intensity level (Io=0,36 mW/cm^2) and UV dose (136.08 mJ/cm^2) was used, only 5.43 ±0.01 log cfu/ml and 5.04 ±0.03 log cfu/ml reduction were achieved in the samples inoculated with the low (5.50 ± 0.107 log cfu/ml) and the high (7.33 ± 0.013 log cfu/ml) initial load, respectively (Table 4.3, Figure 4.11 and 4.13). As a result, this level was not enough to inactivate all *S. cerevisiae* in the pasteurized grape juice especially in the case of high initial microbial load and an incomplete inactivation was obtained. Additionally, the logarithmic reduction was lower for the samples having high initial load than the one inoculated with low number of microorganisms. The reason of this might be due to shadowing effect caused by high number of microorganisms preventing the UV light to reach each microorganisms found in the medium. The survival curves were not linear exhibiting tailing regions at the high UV dose levels (Figure 2.6) This finding was in good aggrement with those reported by Karel and Lund (2003).

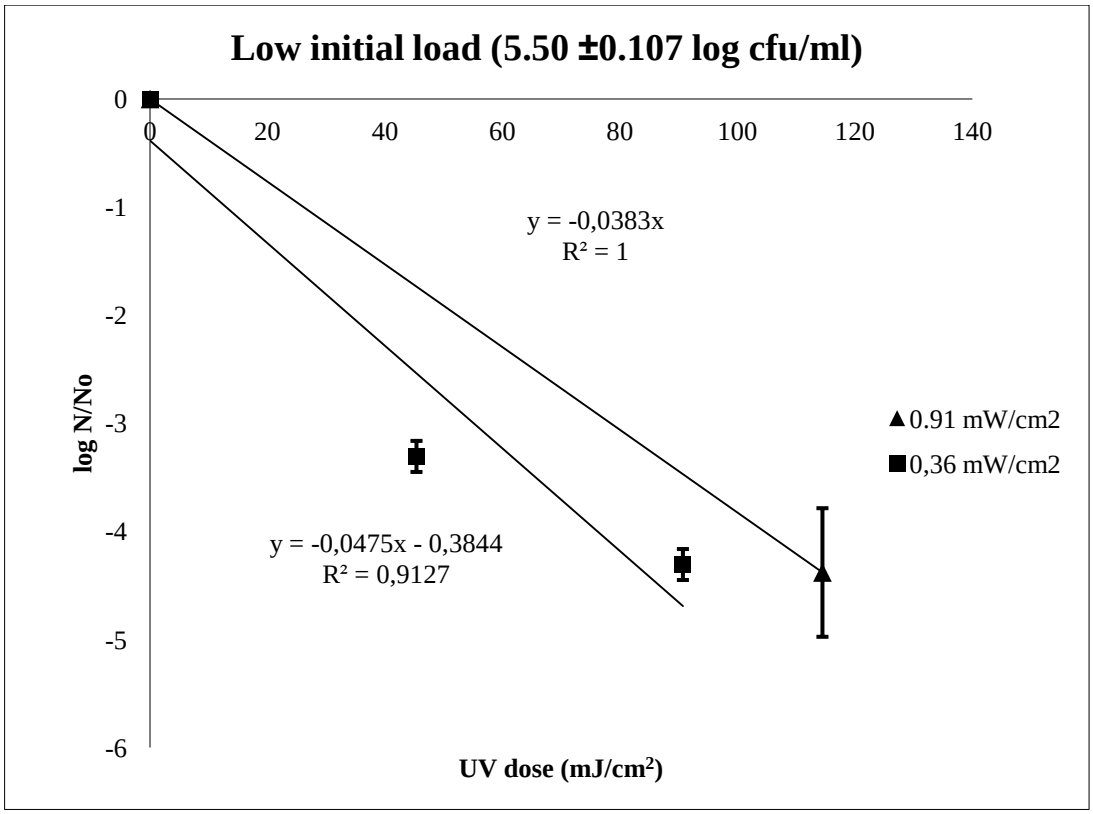


Figure 4.11. The effect of UV-C dosage on *S.cerevisiae* in the pasteurized white grape juice with 5.50 ±0.107 log cfu/ml initial microbial load.

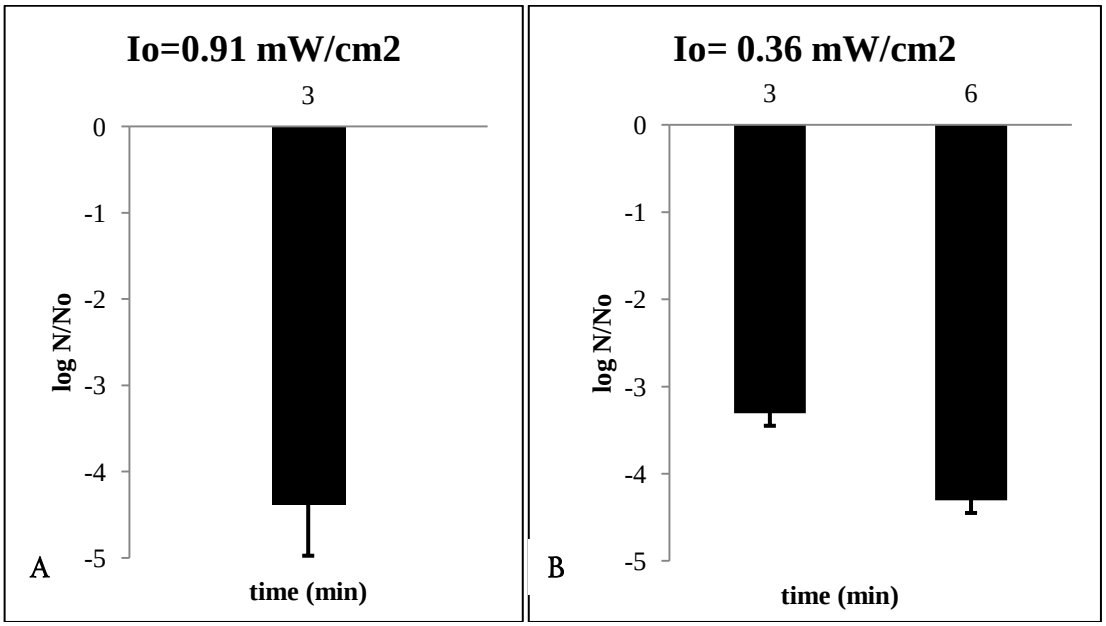


Figure 4.12. Exposure time curves for *S.cerevisiae* in the pasteurized white grape juice with 5.50 ±0.107 log cfu/ml initial microbial load. A. Io=0.91 mW/cm^2, B. Io= 0.36 mW/cm^2.

On the other hand, in the samples having higher initial load (7.33 ± 0.013 log cfu/ml), the complete inactivation (7.33 ±0.03 log cfu/ml) was achieved at the low intensity level (Io=0.36 mW/cm^2) if the higher UV dose level (181.44 mJ/cm^2) and longer exposure time (12 min) was employed (Table 4.3).

Figure 4.12 and 4.14 demonstrates the logarithmic reductions as a function of exposure time. After exposure time of 6 minutes, *S.cerevisiae* strains were completely inactivated at Io=0,91 mW/cm^2 in the both of juice samples having 5.50 ±0.107 log cfu/ml and 7.33 ±0.03 log cfu/ml *S. cerevisiae*. At the Io=0,36 mW/cm^2 UV intensity level, 9 minutes were enough to inactivate all of microorganisms in the juice having 5.50 ±0.107 log cfu/ml microbial load while 12 minutes was necessary for complete inactivation of *S. cerevisiae* in the juice having 7.33 ±0.03 log cfu/ml initial load.

$D_{(10)}$ value (mj/cm^2) that are known as the UV dose for one log reduction were calculated for each intensity level (Table 4.4). D_{10} values for different levels of initial load (5.50 ±0.107 log cfu/ml and 7.33 ±0.03 log cfu/ml) were calculated as 26.11 mJ/cm^2 and 27.55 mJ/cm^2 at the UV intensity of 0.91 mW/cm^2. This means that the high initial microbial load required more UV dosage for one log reduction of microorganisms because of the shadowing effect of the microorganism. Additionally, the juice having high content of microbial load (7.33 ±0.03 log cfu/ml) required less $D_{(10)}$ value at the high intensity level ($D_{(10)}$= 27.55 mJ/cm^2) compared to the case where the low UV intensity was employed ($D_{(10)}$= 27.93 mJ/cm^2). Because microorganisms were exposed to higher dose at the high UV intensity level. Literatures reported that D values of *S. cerevisiae* in the water was reported as 6,6 mJ/cm^2 (Table 2.7). This value is lower than D values found in this study because of the absorbance difference between white grape juice and water.

k values (cm^2/mJ) known as inactivation rate constant were calculated from the slope of the log N/No versus UV dose curve based on the Chick's model (Equation 3.7) (Table 4.4). It is an important parameter to understand sensivity of microorganisms. It shows UV susceptibility of microorganisms e.g. microorganisms that are more sensitive to UV have high k values (Kowalski, 2009; Unluturk, et al. 2008). The juice having 5.50 ±0.107 log cfu/ml microorganisms were more sensitive to UV light at the both intensity level (k= 0.0383, 0.0475) than the juice having microbial load of 7.33 ±0.03 log cfu/ml (k= 0.0363, 0.0358). Murakami, et al. (2006) studied the effect of three different UV intensity on *E.coli* K12 in caramel 13% solution (Io= 0.5; 1 and 2

mW/cm^2). They indicated that sensivity of microorganisms were increased by raising of intensity level. The highest inactivation rate constant (k) was provided at the highest intensity level of 2 mW/cm^2 with 0.4105 than others (k values were 0.2368 for 0.5 mW/cm^2 and 0.2985 for 1 mW/cm^2). For the case where 7.33 ±0.013 log cfu/ml initial load was used, *S. cerevisiae* strains were more sensitive to UV light at higher intensity (Io=0.91 mW/cm^2) with k value of 0.0363 than the one where k was equal to 0.0358 at Io= 0.36 mW/cm^2 . This finding was in good aggrement with Murakami et al. (2006). But the results obtained for the case of low initial load did not show the same trend. This might be because UV dose curve for low initial load was in sigmoidal shape and linear model was not correctly fit to describe data.

In summary, the initial load and the incident UV intensity provided on the surface of the samples have great impact on the inactivation efficiency of UV irradiation process.

Table 4.4. Decimal reduction dose ($D_{(10)}$) and inactivation rate constants (k) of pasteurized white grape juice samples in the Bench Top UV study.

Initial microbial load (cfu/ml)	**UV intensity (mW/cm^2)**	**k (cm^2/mJ)**	**$D_{(10)}$ (mJ/cm^2)**	**R- squared value**
5.50 ± 0.107	0.91	0.0383	26.11	1
5.50 ± 0.107	0.36	0.0475	21.05	0.9127
7.33 ± 0.013	0.91	0.0363	27.55	1
7.33 ± 0.013	0.36	0.0358	27.93	0.9368

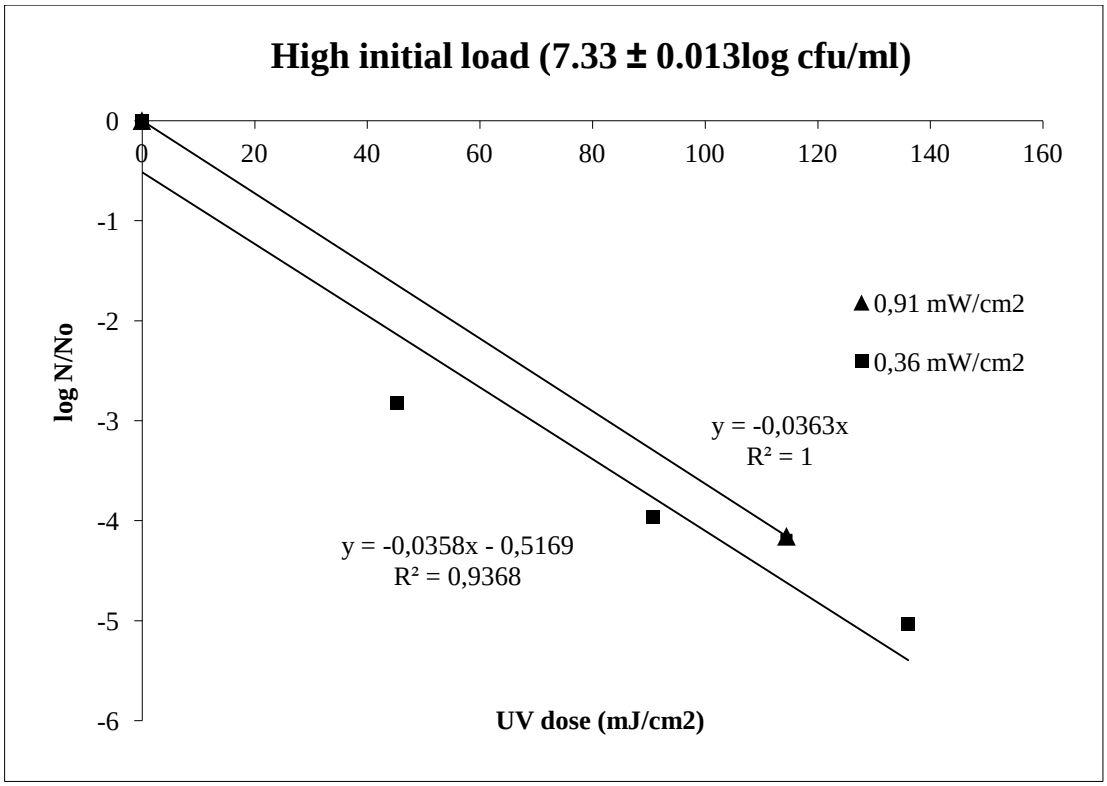


Figure 4.13. The effect of UV-C dosage on *S.cerevisiae* in the pasteurized white grape juice with 7.33 ±0.013 log cfu/ml initial load.

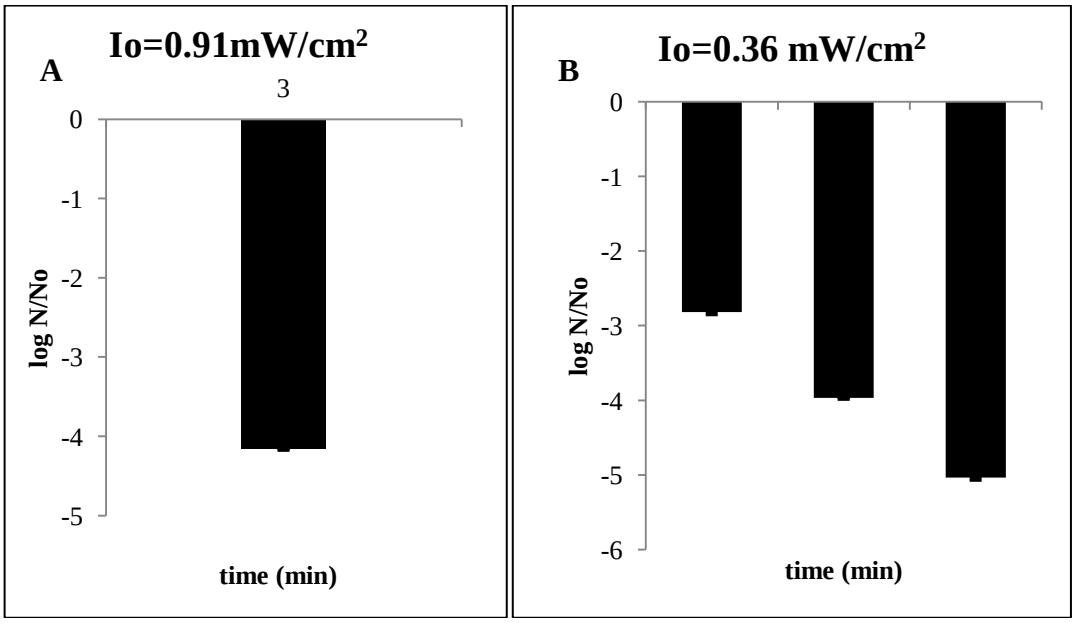


Figure 4.14. Exposure time bars for *S.cerevisiae* in the pasteurized white grape juice with 7.33 ±0.013 log cfu/ml initial microbial load. A. Io=0.91 mW/cm^2, B. Io= 0.36 mW/cm^2.

4.2.1.2. Fresh Squeezed White Grape Juice

Bench top UV biodosimetric study was performed at the highest UV intensity (Io= 0.76 mW/cm^2) that can be achieved by placing the petri dish in a distance very close to UV source in the collimated beam UV apparatus. Different exposure times and consequently different UV dose levels were studied. The final inoculation rate was adjusted to 5.98 ±0.026 log cfu/ml for yeasts and 7.94 ±0.039 log cfu/ml for lactic acid bacteria by diluting naturally spoiled white grape juice with freshly squeezed juice. Yeasts were counted on PDA (Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) and lactic acid bacteria were counted on MRS Agar (Merck, Darmstadt, Germany) by using spread plating method. UV Intensity supplied from UV lamps on the juice surface was measured as Io= 0.76 mW/cm^2 which was less than intensity level provided on the pasteurized white grape juice surface. This was arised from the high absorbtive and turbid structure of the freshly squeezed juice. Applied UV dosages and logarithmic microbial reductions achieved at these dosages were listed in Table 4.5.

Table 4.5. Log reductions of spoilage microorganisms in the fresh squeezed white grape juice at a constant UV-C intensity of Io=0.76 mW/cm^2.

		Time (min)	**UV dosage (mJ/cm2)**	**Log reduction (log No/N)**
Spoilage microorganisms	**yeasts**	6	70.56	1.60 ±0.01
		12	141.12	2.65 ±0.075
		18	211.68	2.86 ±0.01
		24	282.24	3.00 ±0.16
	lactic acid bacteria	6	70.56	2.27 ±0.03
		12	141.12	3.51 ±0.01
		18	211.68	3.97 ±0.001
		24	282.24	4.32 ±0.03

According to the Table 4.5, total UV exposure times of 24 minutes provided less inactivation of yeasts (3.00 ±0.16 log cfu/ml) than lactic acid bacteria (4.32 ±0.03 log cfu/ml) at the same UV dosage of 282.24 mJ/cm^2 (Figure 4.17). Tran and Farid (2004) obtained 0.53 and 0.35 log reduction of lactic acid bacteria and yeasts at the UV dose of 73.8 mJ/cm^2 in the fresh orange juice while 70.56 mJ/cm^2 UV dose provided 2.27 and 1.60 log cfu/ml for yeasts and lactic acid bacteria in our study due to more clear structure of the white grape juice.

In comparing with pasteurized white grape juice study, inhibitive effects of suspended particles and absorbance of the juice were noticed in the fresh squeezed juice (Figure 4.15 and 4.16). Suspended particles in its composition blocked the UV light and the light did not penetrate effectively to juice. Additionally, fresh squeezed juice was also more absorptive to UV light and evantuated in less exposure of UV dosages on the samples.

Similar study as UV inactivation of spoilage microflora in the apple juice were performed by Noci et al. (2008). The freshly squeezed apple juice with 1,63 cm juice depth and an initial microbial load of 10^9 CFU/ml was exposed to 30 mW UV light source for 30 minutes in a batch top UV system. Their study resulted in 2.2 log reduction in spoilage. However, in our study 4.32 ±0.03 log cfu/ml reduction was achieved in spoilage bacteria (lactic acid bacteria) after employing UV light after 24 minutes of exposure time (Figure 4.17). Higher inactivation was obtained by using 0,153 cm juice depth allowing penetration of UV light to the juice sample more than their study. Another reason of achieving more logarithmic reductions may be less count of initial spoilage bacteria (7.94 ±0.039 log cfu/ml) used in this study.

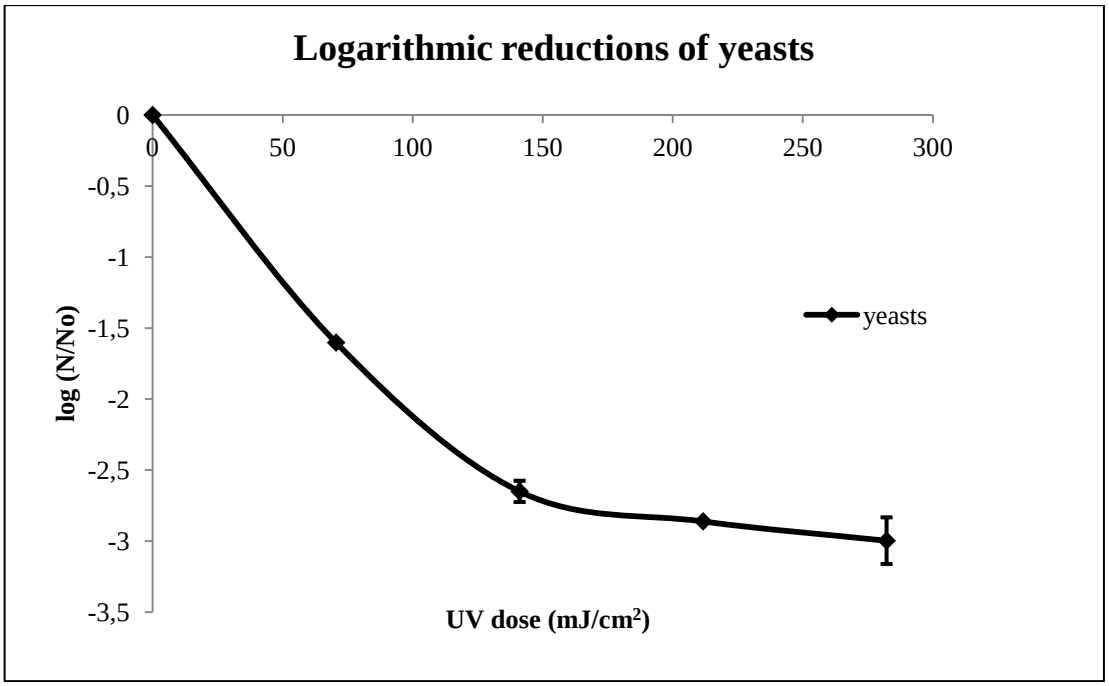


Figure 4.15. The effect of UV-C dosage on yeast counts in the fresh squeezed white grape juice at Io =0.76 mJ/cm^2 (Tray 1), 5.98 ±0.026 log cfu/ml initial microbial load.

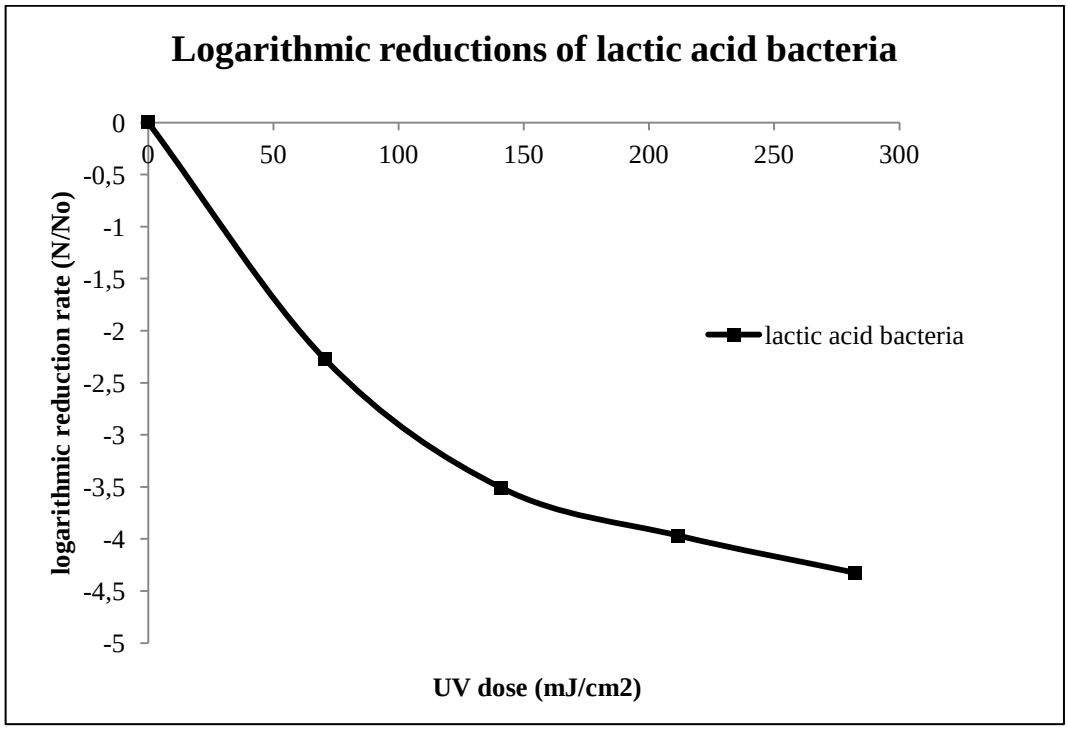


Figure 4.16. The effect of UV-C dosage on lactic acid bacteria counts in the fresh squeezed white grape juice at Io =0.76 mJ/cm^2 7.94 ±0.039 log cfu/ml initial microbial load.

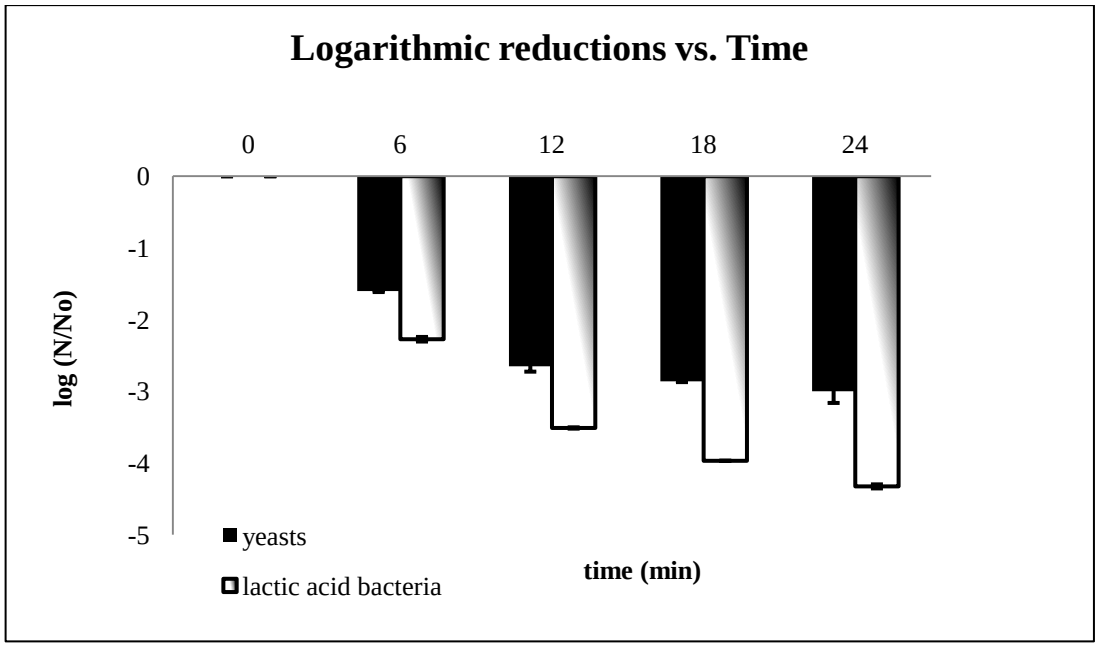


Figure 4.17. Exposure time bars for yeasts and lactic acid bacteria in the fresh squeezed white grape juice at the Io =0.76 mJ/cm^2, 5.98 ± 0.026 log cfu/ml initial yeasts and 7.94 ±0.039 log cfu/ml initial lactic acid bacteria.

Principally, the inactivation curve of spoilage flora was sigmodial for fresh squeezed white grape juice because of tailing effects of suspended particles in its composition (Karel and Lund, 2003) (Figure 4.15 and 4.16). However, in order to apply the linear model to calculate kinetic parameters related to UV inactivation such as $D_{(10)}$ and k values,, the first several logarithmic reduction data were used (Figure 4.18). According to the linear model, there was a superior correlations between logaritmic reductions and UV dosages with R-squared values of 1 and 0.9932 (Table 4.6).

Table 4.6. Decimal reduction dose ($D_{(10)}$) and inactivation rate constants (k) of fresh squeezed white grape juice samples in the Bench Top UV study.

Spoilage microflora	**UV intensity (mW/cm^2)**	**k (cm^2/mJ)**	**$D_{(10)}$ (mJ/cm^2)**	**R-squared values**
Yeasts	0.76	0.0228	43.86	1
Lactic acid bacteria	0.76	0.0296	33.78	0.9932

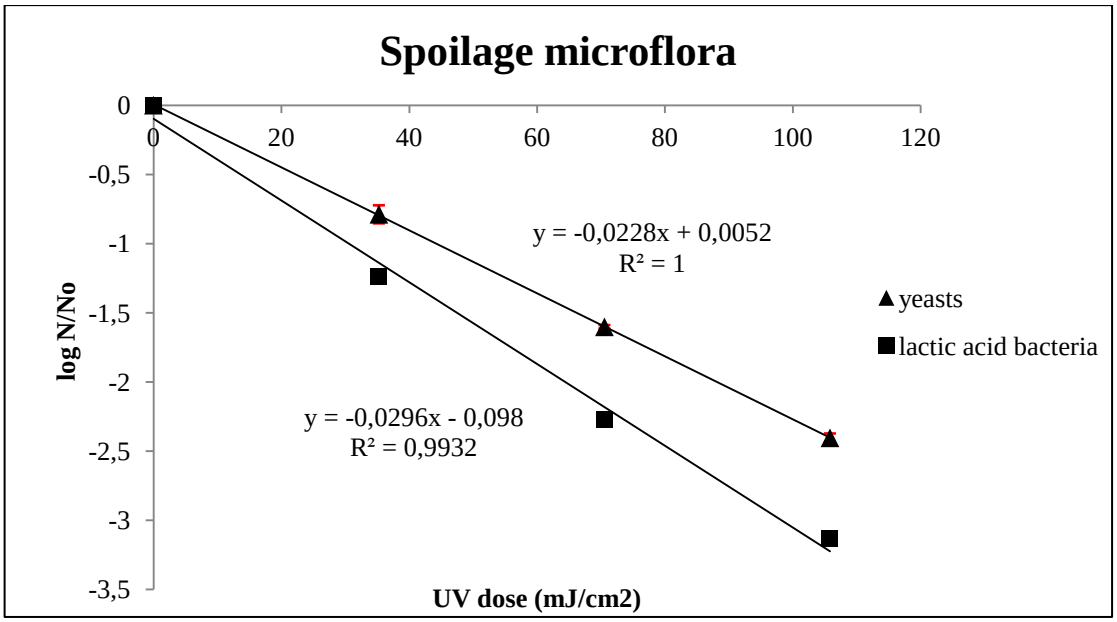


Figure 4.18. Linear model of fresh squeezed white grape juice with the initial yeasts of 5.98 ±0.026 log cfu/ml and lactic acid bacteria of 7.94 ±0.039 log cfu/ml.

It as reported that yeasts were more resistant microorganisms to the UV light than lactic acid bacteria due to their DNA structure (Barbosa-Canovas, 2005). $D_{(10)}$ values of yeasts and lactic acid bacteria in Table 4.6 indicated that yeasts required more UV dose for one log reduction (43.86 mJ/cm2) than lactic acid bacteria (33.78 mJ/cm2). Similarly, k value of lactic acid bacteria (0.0296 cm^2/mJ) was higher than yeasts (0.0228 cm^2/mJ) due to higher sensivity. Tran and Farid (2004) studied in spoilage microflora of the fresh squeezed orange juice. They found higher $D_{(10)}$ values that were 87 and 119 mJ/cm^2 for lactic acid bacteria and yeasts than in our study because orange juice is more turbid liquid than white grape juice.

4.2.2. Continuous Flow UV Inactivation Results of White Grape Juice Samples

In the conventional bench top scale UV systems, high turbid liquid foods such as fresh squeezed fruit juices have non-uniform dispersion and UV-C light does not penetrate completely because of the suspended particles. Additionally, they are highly absorptive liquid foods and the efficiency of the UV beams is lower when comparing to clear liquids. Therefore, continuous flow UV reactor systems are designed for high absorbance liquid foods such as fresh fruit juices to overcome the adverse effects of the

high absorbance and turbidity (Koutchma, et al. 2009). Significant factors which affect the continuous flow UV reactor systems are absorbance of the fluids, flow behaviour of the fluids in the UV reactor, light intensity distribution and reactor design in the system (Koutchma, et al. 2004). In this study, white grape juice samples were cycled eight times in a continuous flow UV reactor system in order to investigate the efficiency of this system on the clear juice (pasteurized juice) and freshly squeezed opaque juice containing particles (fresh juice). Thus, the effect of particles on the continuous flow UV inactivation system was studied and compared with the literature.

Table 4.7. Flow and UV parameters for pasteurized and fresh squeezed white grape juice samples.

White grape juice	**Pasteurized**			**Fresh squeezed**		
Flow rates	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Flow rate (ml/min)	820	1520	2280	774	1512	2085
Velocity (cm/s)	0.67	1.25	1.88	0.64	1.24	1.72
Re number	184.36	340.83	512.60	163.87	320.57	442.76
T_{uv} (for 1 cycle) (s)	182	103	70	180	126	77
T_{uv} (total 8 cycle) (min)	24.27	13.73	9.33	24	16.8	10.26
Total UV dose (mj/cm^2) (after 8 cycles)	56.06	23.07	13.66	19.30	19.96	8.00

The flow parameters of the pasteurized and fresh squeezed white grape juice and total UV doses that are applied after cycling eight times in a continuous flow UV reactor were listed in Table 4.7. It can be said that all continuous flow UV experiments were carried out in the laminar flow regime due to low Re numbers. According to the Table 4.7, UV dose calculated from the inactivation of grape juice in the continuous flow system was generally decreased by increasing the flow rates. Because UV cycling time in the system diminished by increasing flow rate and resulted in lower UV doses. Microbial inactivation and quality parameters of the juice samples were analyzed in the section 4.2.2.1., 4.2.2.2 and 4.2.2.3, broadly.

4.2.2.1. Microbiological Results of Pasteurized White Grape Juice

Inoculation study for the pasteurized white grape juice were done according to the section 3.3.1.1. *S.cerevisiae* strains which were cultivated in the broth were centrifugated and strains were inoculated to the 9000 ml clear juice in order to provide final inoculation rate around 5.90-5.95 log cfu/ml. After the adjustment of initial microbial load, juice were poured into the stainless tank of the continuous flow UV reactor system which was cleaned previously and pumped through the system to succeed in UV disinfection of the juice. Before the disinfection study, volumetric flow rates were measured as ml/min by using a chronometer for each level of the pump frequency (20, 35 and 50 Hz). Eight cycles were selected and a constant holding time of 5 minutes in the collection tank was allowed to achieve complete inactivation of juice circulating in the system. The cycling times were different for each three level of flow rates as low (820 ml/min), medium (1516 ml/min) and high (2280 ml/min) and logarithmic reductions of *S. cerevisiae* were measured from microbiological counts on the petries after each cycle. *S. cerevisiae* strains were counted on PDA (Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) at 25 ˚C for 2-5 days. Total UV dose at each flow rate was calculated from the absorbance of the juice samples and total UV times after eight cycles. Therefore, disinfection of *S. cerevisiae* in the clear white grape juice was compared in terms of the flow rates.

When low flow rate at 820 ml/min was considered, 3.39 ±0.044 log cfu/ml reductions were achieved for *S. cerevisiae* after eight cycles which took up 24.27 min UV exposure time (Table 4.7, Figure 4.19). Light intensity at the surface of the juice (Io) was recorded as 0.55 mW/cm^2 and total UV dosage was found as 56.06 mJ/cm^2 in the continuous flow system (Figure 4.20). The same reduction of *S. cerevisiae* (3.39 ±0.044 log cfu/ml) was achieved at 89.45 mJ/cm^2 and 46.25 mJ/cm^2 from the graph of the Bench top UV study at 0.91 and 0.36 mW/cm^2 (Figure 4.11). UV penetration depth of the juice was larger (5.08 cm) in the continuous flow system, compared to the Bench Top UV system where path length was 0.153 cm. But reasonable UV dose values were obtained for continuous sytem by reading off the bench top UV dose curves. However, 24.27 min were required in the continuous system whereas shorter times such as 3 min

and 6 min were needed in the Bench top system to reach the same UV dose values (Table 4.7, Figure 4.16).

Temperature was also controlled before and after eight cycles. A little fluctuations of microbial reductions were observed from the bars of Figure 4.19. The reason may be that little temperature increase which was detected as 5.5 ˚C during the disinfection of the juice could be the reason of microbial growth, marginally. Microbial log reductions of *S. cerevisiae* and UV dosages in pasteurized grape juice during the UV irradiation at the low flow rate was seen in Figure 4.20. There was a good correlation between log reduction and UV dose values with r^2 value equal to 0.9877 when linear regression model was applied.

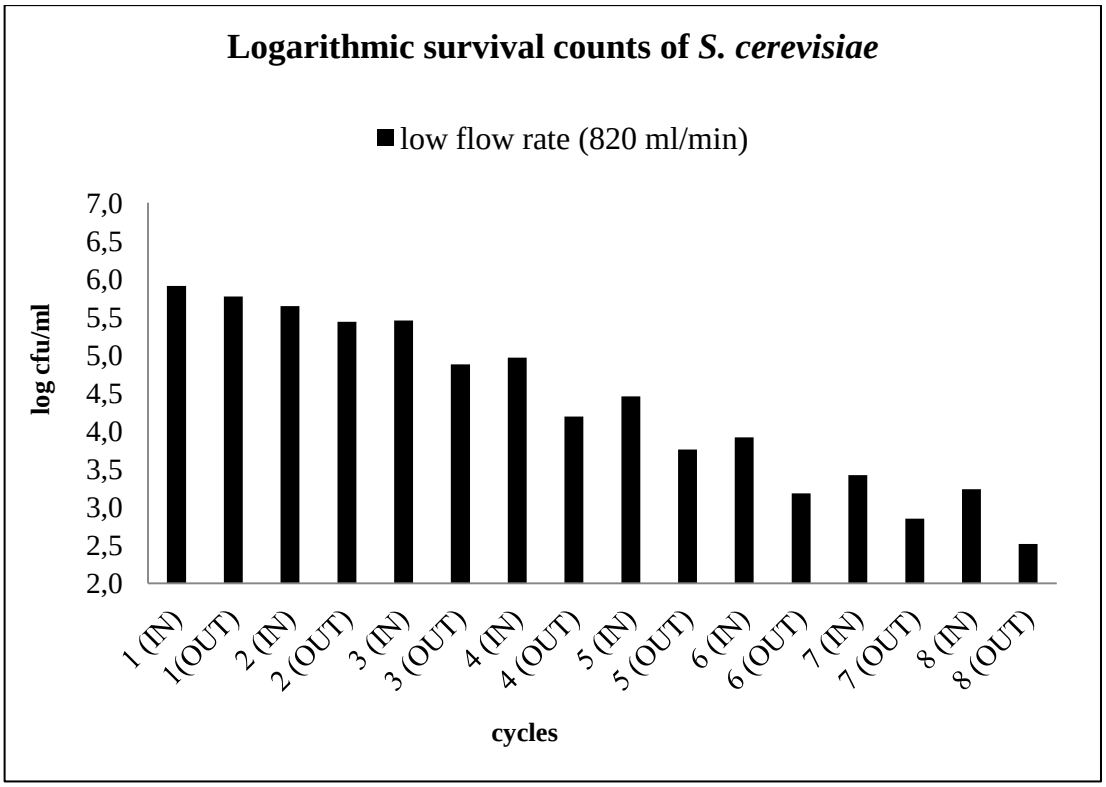


Figure 4.19. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice by using continuous flow UV reactor with 8 cycle at the low flow rate (820 ml/min).

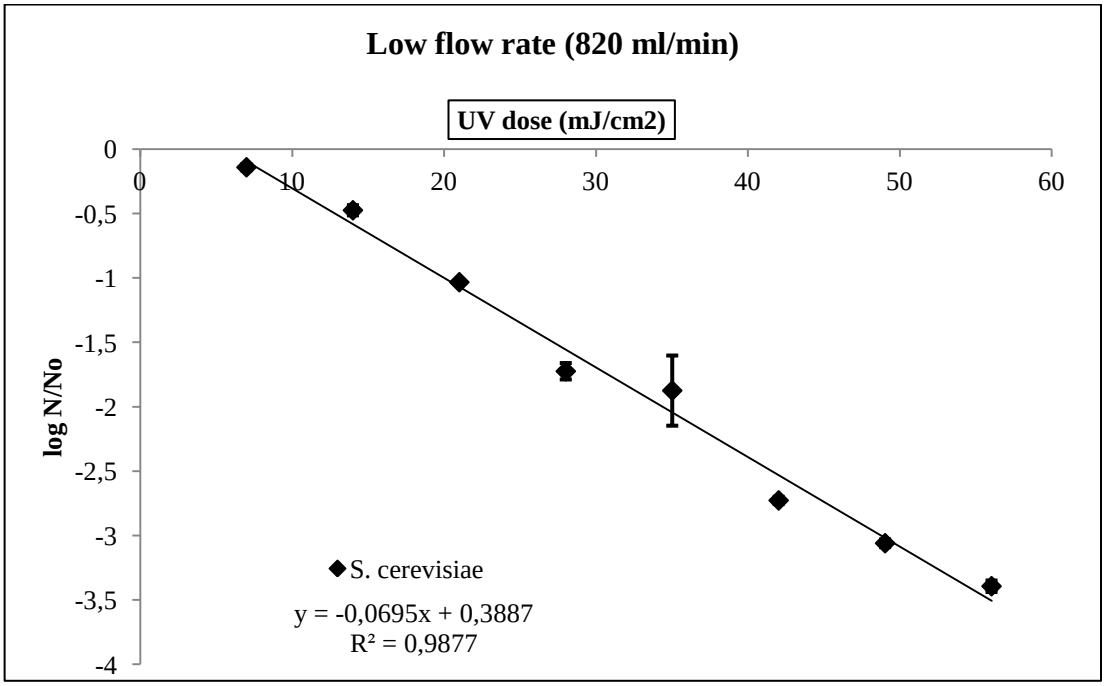


Figure 4.20. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice at low flow rate in the continuous flow UV reactor system.

Logarithmic survival counts of *S. cerevisiae* strains in the pasteurized white grape juice after eight cycles of UV inactivation showed that less inactivation was achieved as 1.874 ±0.006 log cfu/ml in the medium flow rate (1516 ml/min) when comparing to the low flow rate (Figure 4.21). It can be explained that the juice received less UV dosages (23.07 mJ/cm^2) with less total irradiation time (13.73 min) than in the case of low flow rate (56.056 mJ/cm^2; 24.7 min).

UV intensity at the surface was also recorded as 0.37 mW/cm^2 in medium flow rate study. The same logarithmic reduction (1.874 ±0.006 log cfu/ml) was achieved within only 1.7 min by applying 25.57 mJ/cm^2 UV dose at the same intensity (0,36 mW/cm^2) in Bench Top UV study (Figure 4.11). Therefore, UV dose curves obtained by bench top study can give reasonable result for the estimation of UV dose in continuous flow system.

There was no fluctuations observed in the logaritmic reductions (Figure 4.21). This is because temperature increase was only 3.8 ˚C during UV inactivation process at medium flow rate (1516 ml/min).

Although a little shoulder effect was observed at the low UV doses, the linear model gave a high correlation was obtained between applied UV dosages and logarithmic reductions of *S. cerevisiae* strains in the pasteurized white grape juice at medium flow rate (1516 ml/min) because r^2 value was found as 0.985 (Figure 4.22).

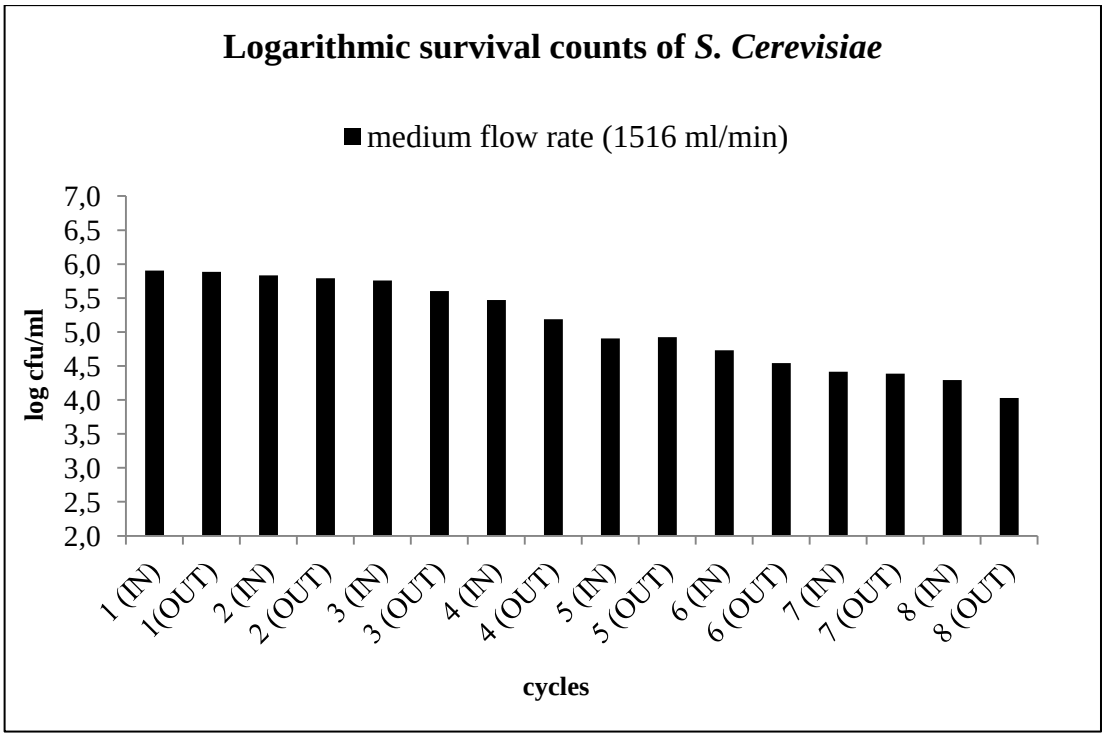


Figure 4.21. Logarithmic reduction of *S. cerevisiae* in the pasteurized white grape juice by using continuous flow UV reactor with 8 cycle at medium flow rate (1516 ml/min).

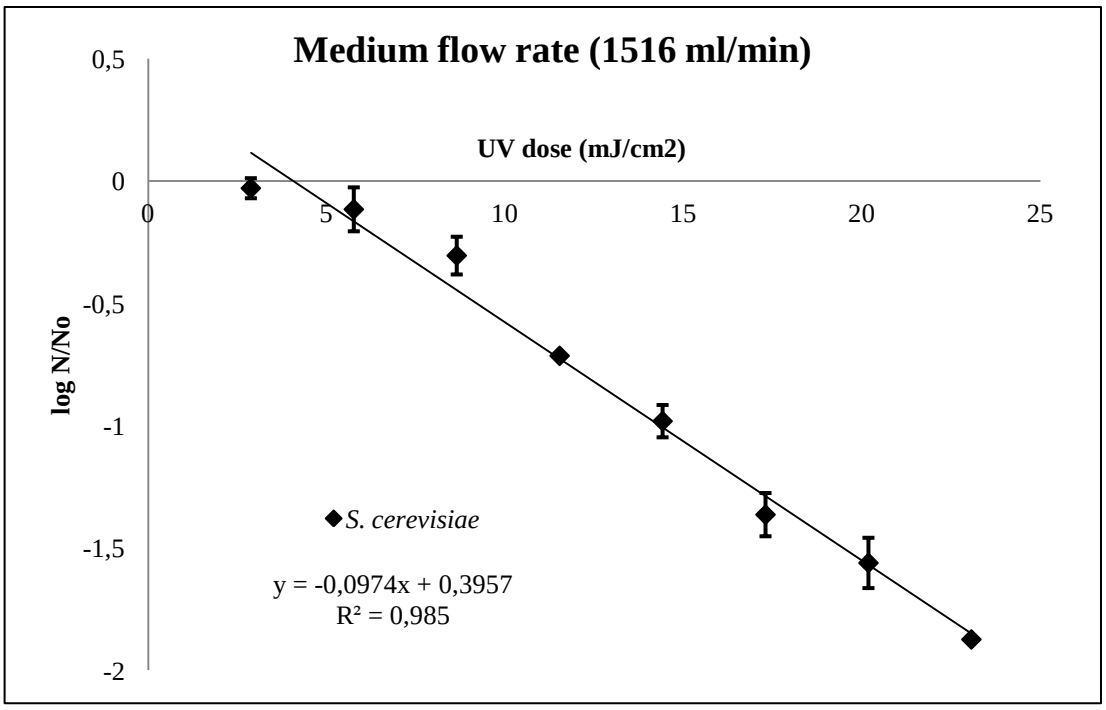


Figure 4.22. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice at medium flow rate of continuous flow UV reactor system.

1.87 ±0.072 log cfu/ml log reductions was obtained at the high flow rate (2280 ml/min) condition where 13.66 mJ/cm^2 UV dose was supplied after 9.33 min exposure time (Figure 4.23). Intensity of the UV light at the surface of the juice was measured as 0.37 mW/cm^2 in the system. Although logaritmic reductions of high flow rate were similar to medium flow rate, applied UV dosage supplied at high flow rate was less than medium flow rate (25.57 mJ/cm^2) because of less total exposure time i.e. 13.73 min. However, some fluctuations of logaritmic *S.cerevisiae* reductions were monitored in the Figure 4.23 because of 4.9 ˚C temperature increase in the system. Re number of the high flow rate was the highest value amoung the flow rates.. Figure 4.24 exhibits a small tailing effect at the high UV doses. But linear model provided a good reasonable correlation between logaritmic reductions and UV dosages for each cycle with r^2 was 0.9785.

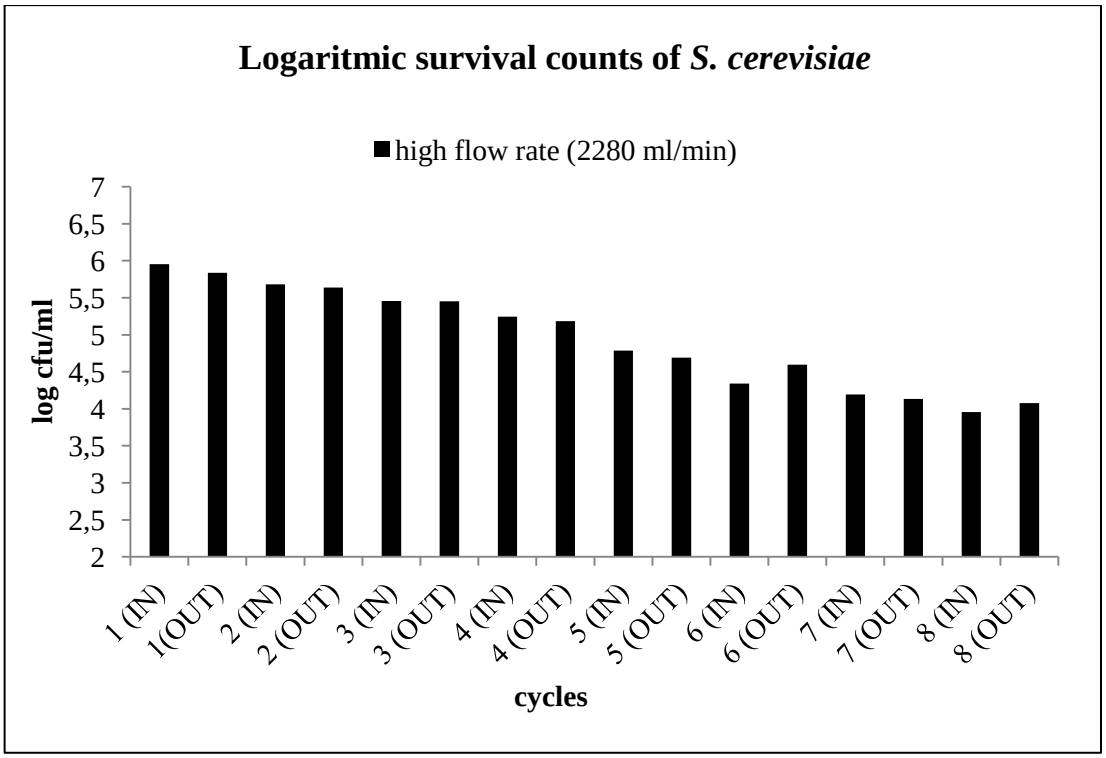


Figure 4.23. Logarithmic reduction of *S. cerevisiae* in the pasteurized white grape juice by using continuous flow UV reactor with 8 cycle at high flow rate (2280 ml/min).

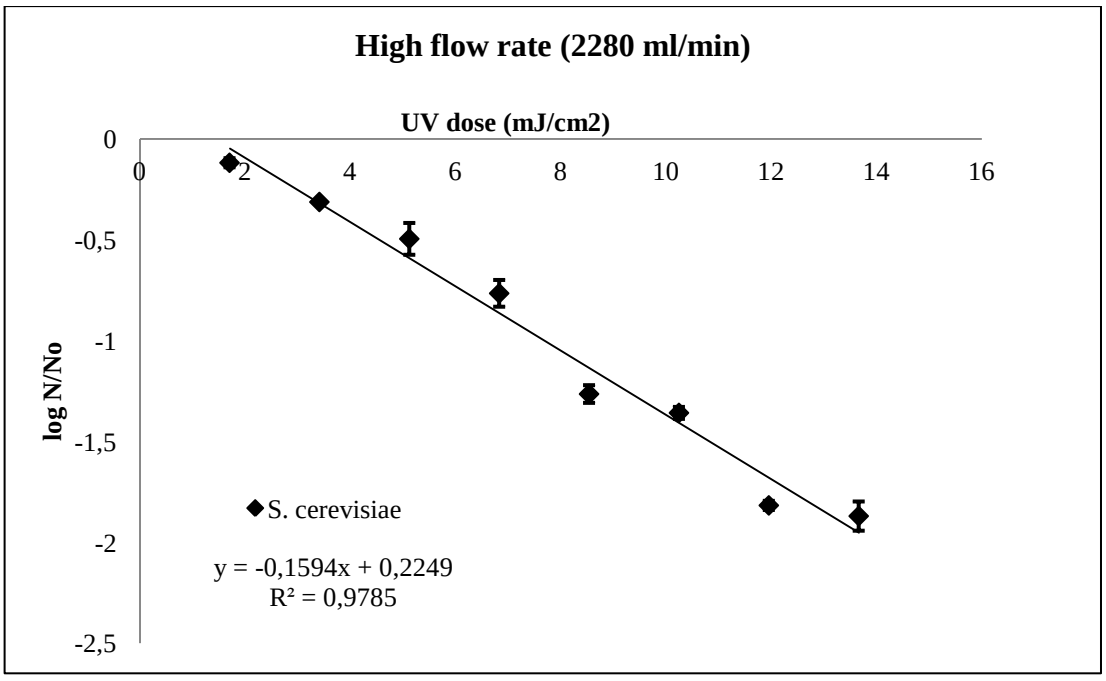


Figure 4.24. Logarithmic reductions of *S. cerevisiae* in the pasteurized white grape juice at high flow rate of continuous flow UV reactor system.

Decimal reduction dose ($D_{(10)}$, mJ/cm^2) and inactivation rate constants (k, cm^2/mj) of each flow rates were calculated from the lineer model of logarithmic reduction versus UV dose graphs (Table 4.8). But the results for mediım and high flow rate were not satisfactory and confusing. Because *S. cerevisiae* strains was found to be more sensitive to UV light at high flow rate which was not truly correct. The more efficient reduction can be expected at the low flow rate because UV light can penetrate to juice better when exposed to longer treatment times. So this results were inconclusive. The reason of this might be the graphs for medium and high flow rates exhibit shoulder and tailing effects. In this case linear model can not give a good fit to data, other models such as Weibull can be used instead.

Table 4.8. Decimal reduction dose ($D_{(10)}$) and inactivation rate constants (k) of pasteurized white grape juice samples in the continuous flow UV reactor system.

Flow rates	**k (cm^2/mJ)**	**$D_{(10)}$ (mJ/cm^2)**	**R- squared value**
Low flow (820 ml/min)	0,0695	14.39	0,9877
Medium flow (1516 ml/min)	0,0974	10,27	0,985
High flow (2280 ml/min)	0,1594	6,27	0,9785

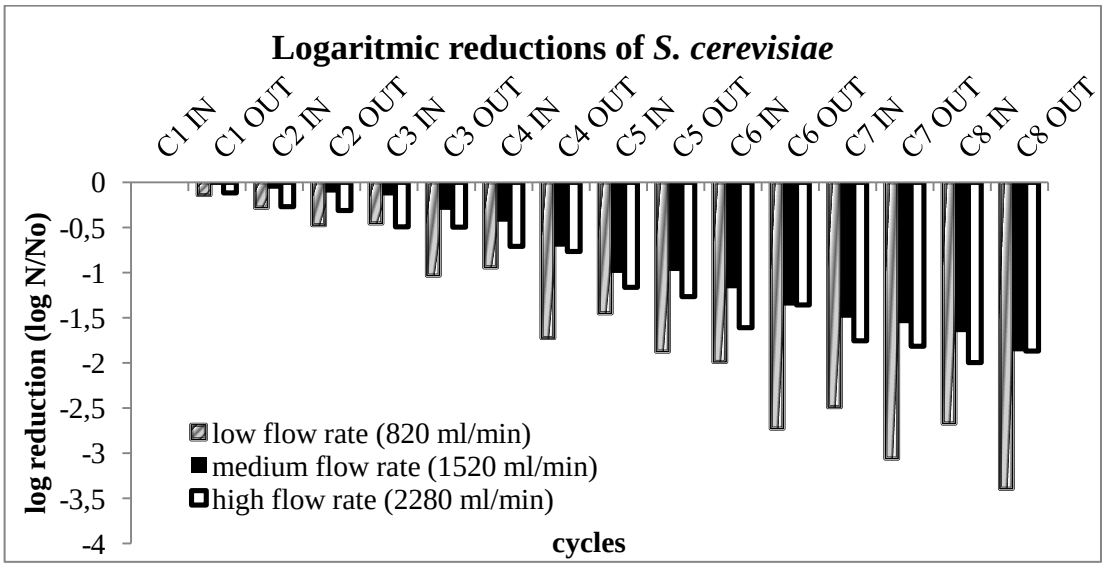


Figure 4.25. Logaritmic reductions of *S. cerevisiae* in the pasteurized white grape juice in the continuous flow UV reactor system with different flow rates as low (820 ml/min), medium (1520 ml/min) and high flow rate (2280 ml/min).

In conclusion, the most effective inactivation (3.39 ±0.044 log cfu/ml) was performed by low flow rate (820 ml/min) with the $D_{(10)}$ value as 14.39 mJ/cm^2 and the lowest k value as 0.0695 cm^2/mJ (Figure 4.25 and Table 4.8).

4.2.2.2. Microbiological Results of Fresh Squeezed White Grape Juice

Final microbial load of fresh squeezed white grape juice for the continuous flow UV study were regulated at 5.5-5.7 log cfu/ml for yeasts around and 7.1-7.8 log cfu/ml for lactic acid bacteria by using fermented juice as explained section 3.3.1.2. Juice were placed into the disinfected stainless steel tank after microbial inoculation and pump was switched on to measure volumetric flow rates. Three different levels of the pump frequency i.e. 20, 35 and 50 Hz were used in this study. The flow rates were measured with a chronometer as 774 ml/min at low frequency, 1512 ml/min at medium frequency and 2285 ml/min at high frequency. Total eight cycle was applied and it was waited for five minutes between each cycle for complete inactivation of all of the white grape juice circulating in the system. Total UV exposure times and dosages were computed after eight cycles and logaritmic microbial reductions of yeasts and lactic acid bacteria of the spoilage microorganisms in the fruit juice were measured after each flow rate. Spoilage microflora of the white grape juice were counted by spreading method on PDA (Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) for the yeasts at 25˚C between 2 and 5 days incubation and pouring method into MRS Agar (Merck, Darmstadt, Germany) for the lactic acid bacteria at 30˚C at 2 days incubation.

Figure 4.26 demonstrates the logaritmic reductions of natural spoilage microflora in freshly squeezed white grape juice after low flow rate (774 ml/min). After eight cycles, 1.54 ±0.035 log cfu/ml yeasts and 1.64 ±0.026 log cfu/ml lactic acid bacteria reductions were detected after 19.30 mJ/cm^2 UV dosages. Yeasts were less inactivated because they are more resistant to UV light than bacteria (Barbosa-Canovas, 2005). Juice temperature was increased 8 ˚C during the experiments because low flow rate results in more total exposure time (24 min). Intensity at the juice surface were measured as 0.45 mW/cm^2 in the reactor. The logaritmic reductions of spoilage microorganisms in the freshly squeezed white grape juice were described in terms of UV dosages in Figure 4.27. Although a slight tailing effect and shoulder effect were

seen in UV dose curve for lactic acid bacteria and yeast, linear model was used to define $D_{(10)}$ (mJ/cm2) and k values (cm^2/mJ) in the continuous system providing a reasonable correlations ((r^2) was 0.9815 for yeasts and 0.9223 for lactic acid bacteria).

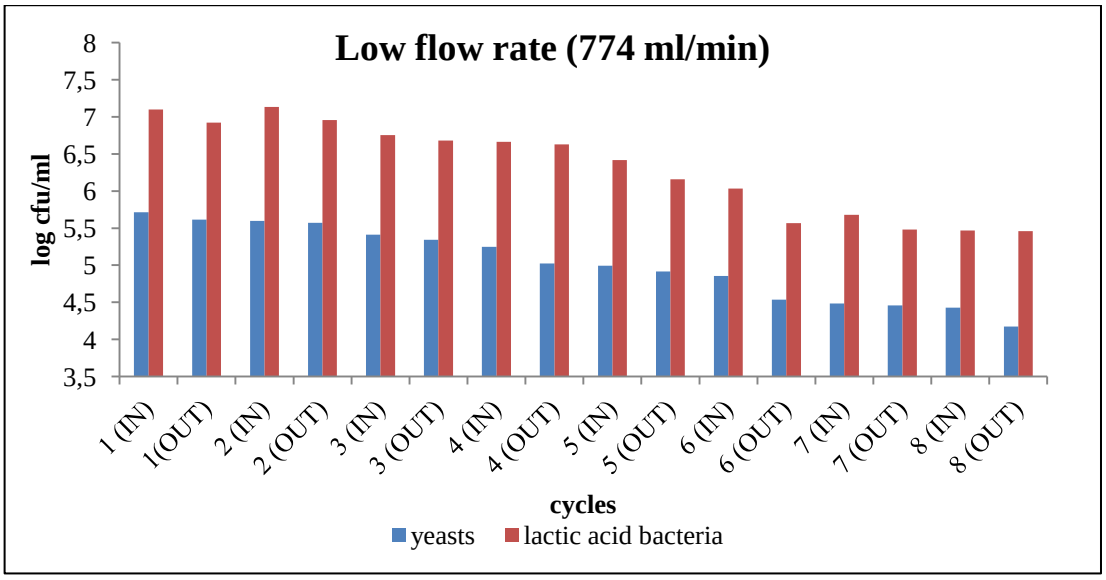


Figure 4.26. Logaritmic reductions of spoilage microflora in the fresh squezed white grape juice by using continuous flow UV reactor with 8 cycle at low flow rate (774 ml/min).

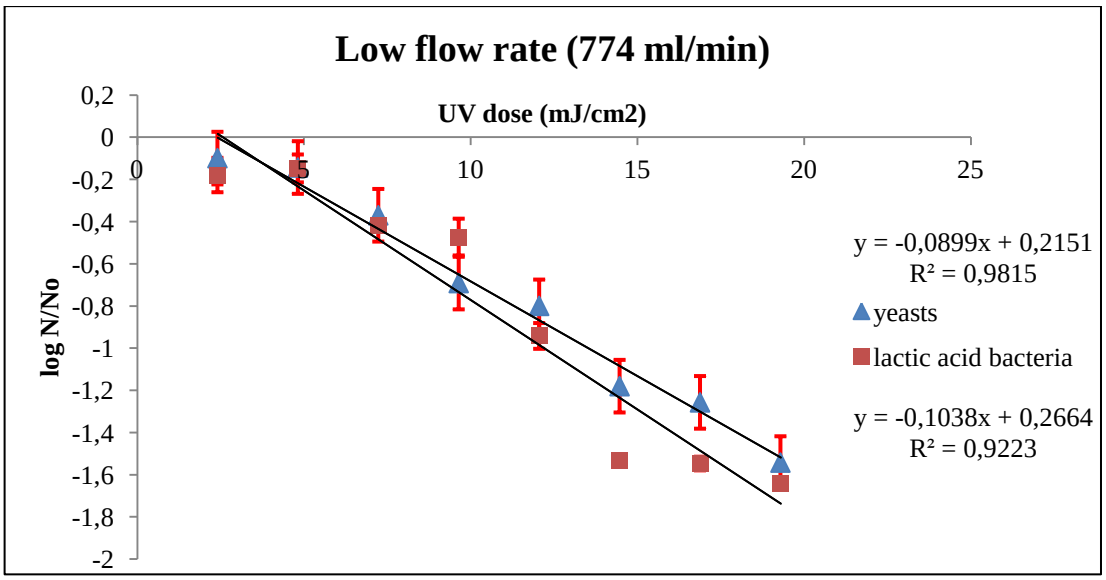


Figure 4.27. Logaritmic reductions of spoilage microflora in the fresh squeezed white grape juice at low flow rate of continuous flow UV reactor system.

At the medium flow rate (1512 ml/min) of fresh squeezed white grape juice, the most effective logaritmic reductions of yeasts and lactic acid bacteria were obtained as 1.70 ±0.028 log cfu/ml and 2.49 ±0.0031 log cfu/ml, respectively (Figure 4.28). The reason was due to having grape juice which has lower turbidity (80.1 ±0.64 NTU) than the juice used in the other treatment conditions (105±2.12 NTU for low flow rate; 113±0.7 NTU for high flow rate). That's why, the highest UV dosage of 19.96 mJ/cm^2 was calculated in the medium flow rate with the 0.70 mW/cm^2 juice surface intensity for 16.8 min total UV treatment time. Additionally, temperature (5.5˚C increment) was better controlled than other treatment conditions.

Although a slight shoulder effect was seen in the UV dose curve (Figure 4.29) there was a good relationship between logaritmic reductions of yeasts and lactic acid bacteria versus UV dose calculations at the medium flow rate (1512 ml/min). When linear regression model was applied (r^2 values of yeasts and lactic acid bacteria were 0.9573 and 0.9901, respectively).

The similar reduction of yeasts (1.70 ±0.028 log cfu/ml) and lactic acid bacteria (2.49 ±0.0031 log cfu/ml) in the continuous flow system could be achieved by Bench Top UV study within aproximately 6 min and 70,56 mJ/cm^2 UV dose (Table 4.5). This higher UV dose was resulted from usage of the grape juice having different optical properties. Thus, in this case Bench Top UV dose curve can not be used for estimation of UV dose in continuous flow system.

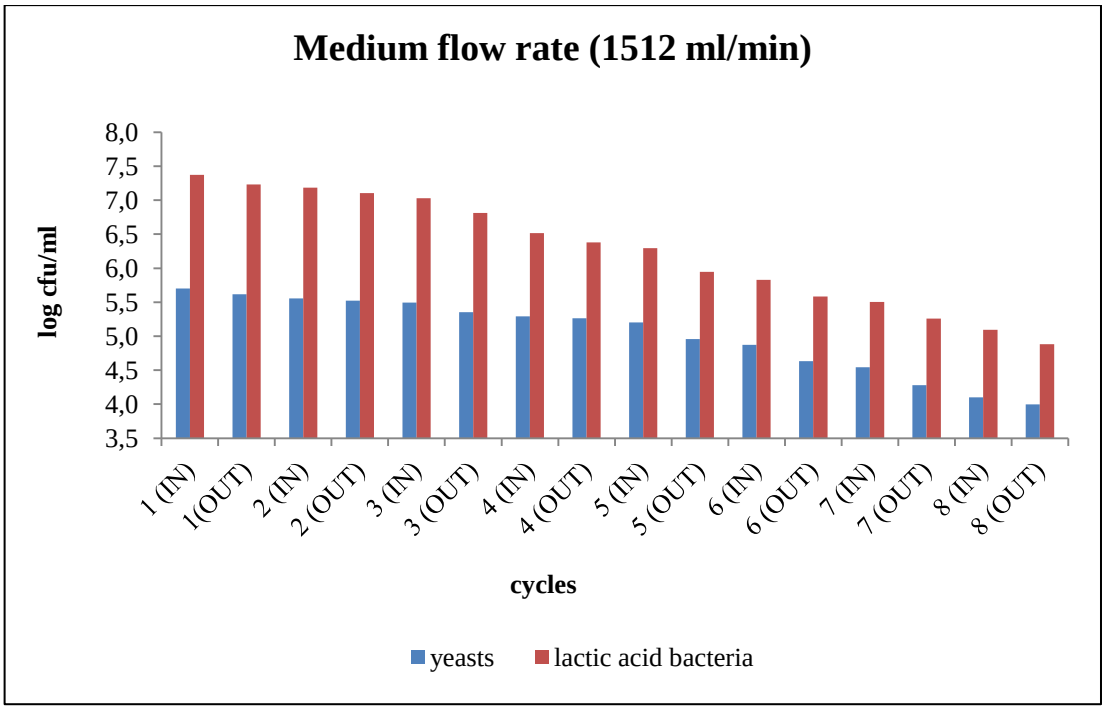


Figure 4.28. Logaritmic reductions of spoilage microflora in the fresh squezed white grape juice by using continuous flow UV reactor with 8 cycle at medium flow rate (1512 ml/min).

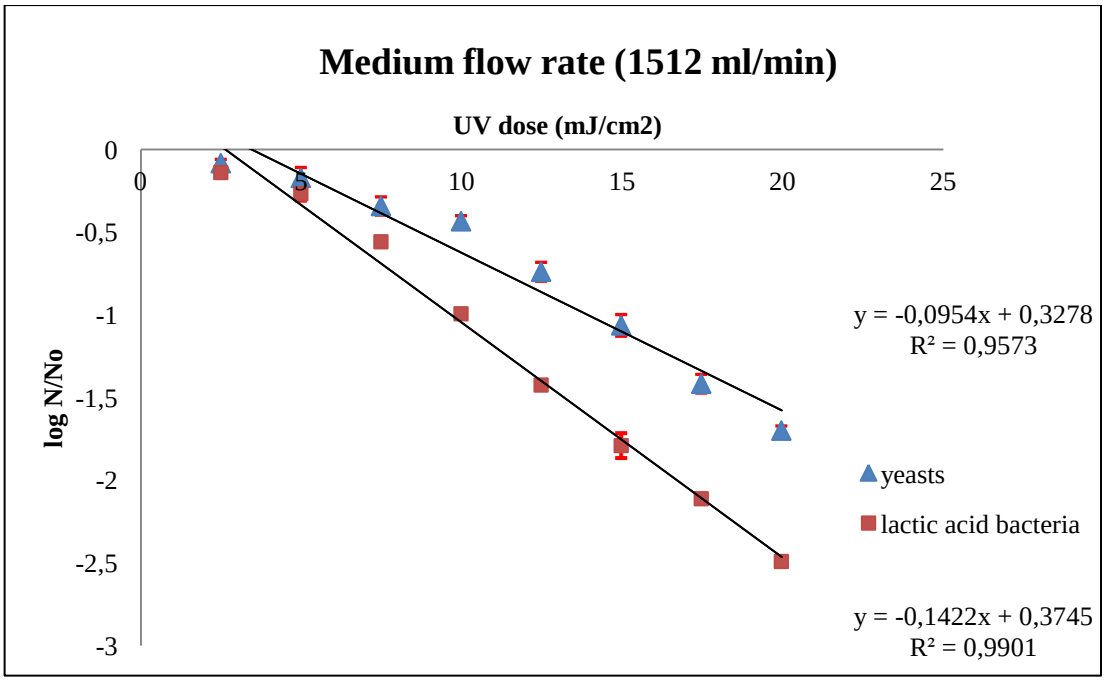


Figure 4.29. Logarithmic reductions of spoilage microflora in the fresh squeezed white grape juice at medium flow rate of continuous flow UV reactor system.

The experiment conducted at the high flow rate (2085 ml/min) was resulted in 0.47 ±0.027 log cfu/ml reduction in yeast count and 1.084 ±0.12 log cfu/ml reduction in lactic acid bacteria count at 8.00 mJ/cm^2 UV dose and 10.26 min total UV exposure time (Figure 4.30). Yeasts were reduced less than lactic acid bacteria due to their DNA strand included less pyrimidine bases and thus more resistant microorganisms to the UV light (Barbosa-Canovas, 2005).

There was some fluctuations occured among the log reductions of each cycle shown in Figure 3.30. Temperature raise after the high flow rate inactivation was 8°C. Number of microorganisms increased slightly during middle of the inactivation study because of the temperature effect and then reduced linearly. Log reductions and UV dose showed linear trend after the middle of the experiment. After the fourth cycle, microorganisms were reduced linearly with r^2 were 0.9639 and 0.9621 (Figure 4.31).

Other than temperature, flow rate can also be effective in the microbial reduction by causing less exposure time. Although temperature increase was the same between low and high flow rate (8°C), microbial reductions were unequal because of the differences of UV dosages (19.30 mJ/cm^2 for low flow rate and 8.00 mJ/cm^2 for high flow rate). Additionally, juice at the high flow rate remained for a little time in the system resulted in less UV dosage during inactivation. Thus, less microbial reductions were observed. Although juice flowed rapidly at high flow rate (2085 ml/min) through the continuous flow UV system, the juice was still in the laminar flow regime with the highest Re number as 442.76 (Table 4.7).

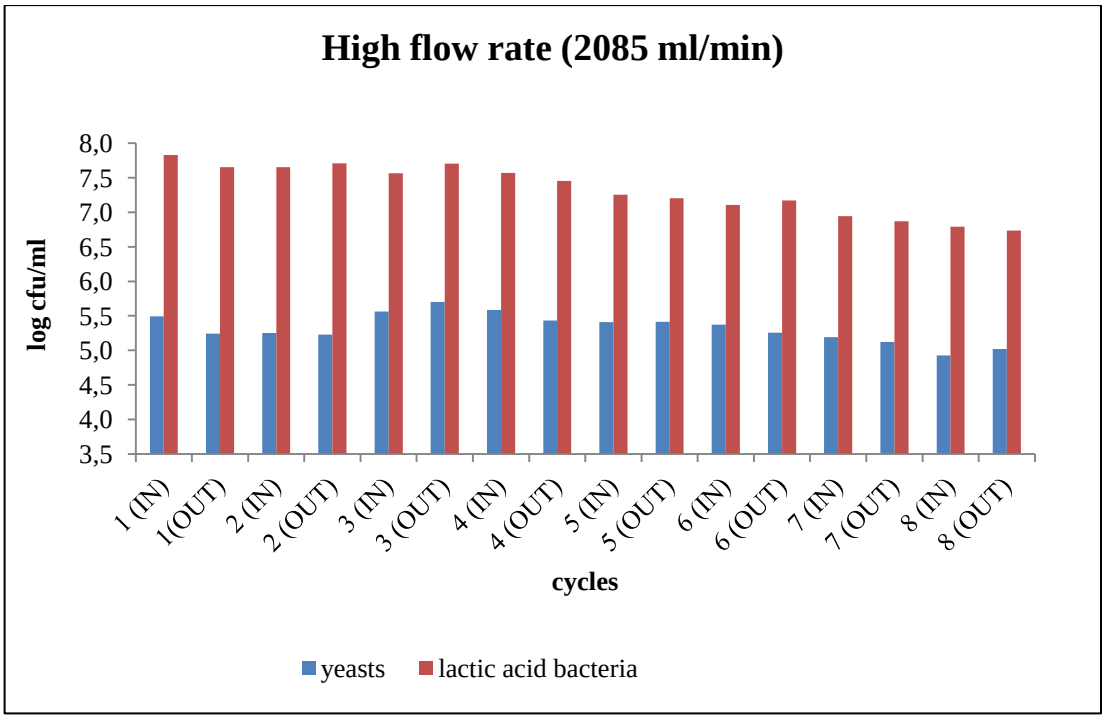


Figure 4.30. Logarithmic reductions of spoilage microflora in the fresh squezed white grape juice by using continuous flow UV reactor with 8 cycle at high flow rate (2085 ml/min).

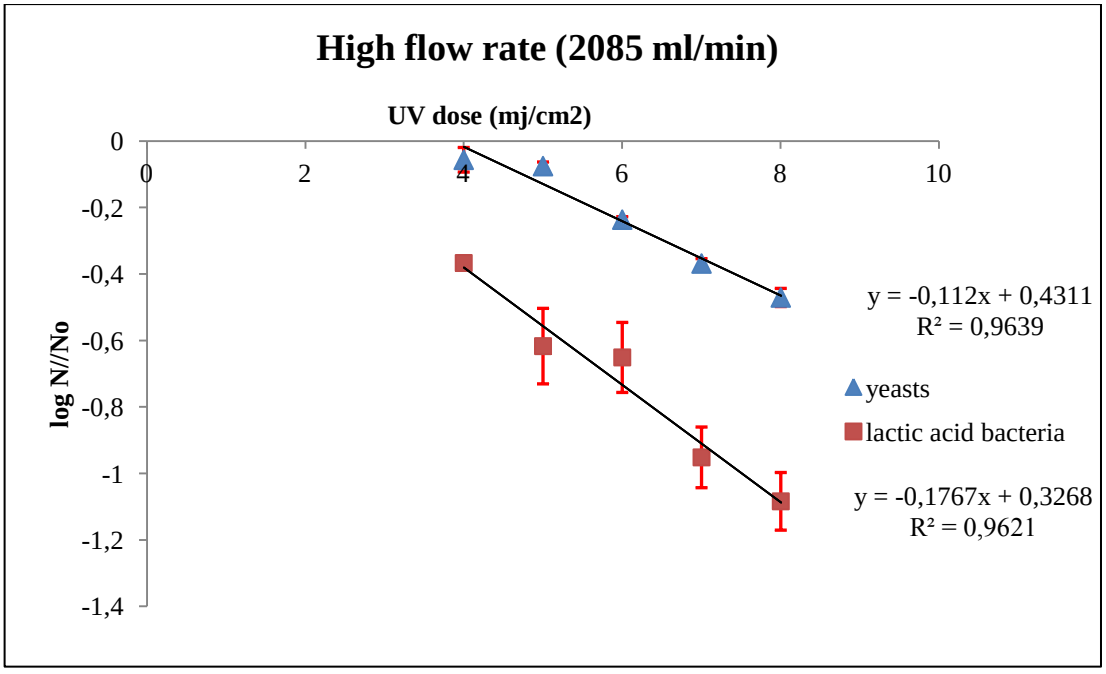


Figure 4.31. Logarithmic reductions of spoilage microflora in the fresh squeezed white grape juice at high flow rate of continuous flow UV reactor system.

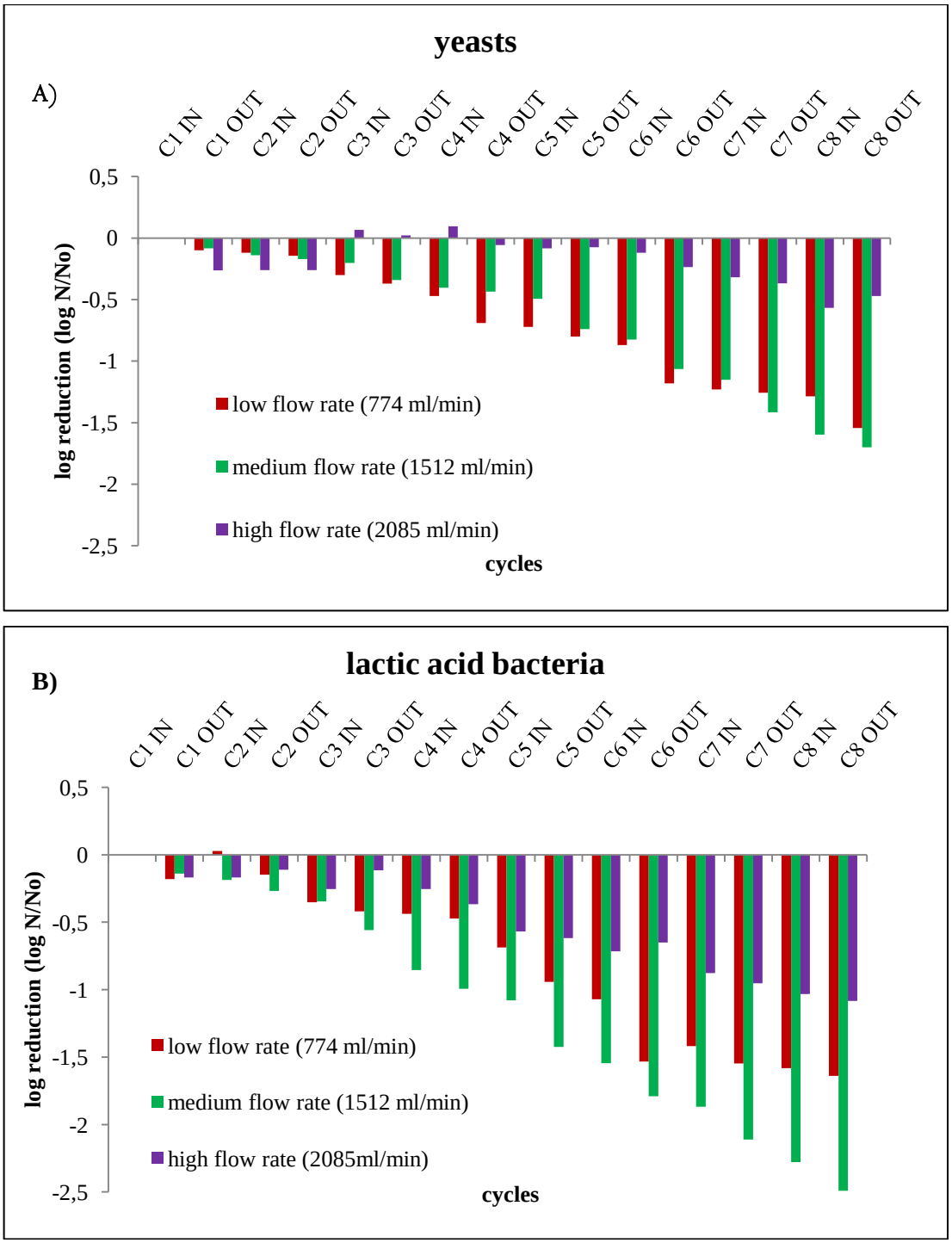


Figure 4.32. Logarithmic reductions of spoilage microflora in the fresh squeezed white grape juice in the continuous flow UV reactor system with different flow rates A. yeast, B. lactic acid bacteria.

Different flow rates caused different logarithmic reductions of microorganisms. Low flow rate (774 ml/min) resulted in higher inactivation of yeasts (1.54 ±0.035 log cfu/ml) and lactic acid bacteria (1.64 ±0.026 log cfu/ml) than high flow rate (2085 ml/min, 0.47 ±0.027 log cfu/ml yeasts and 1.084 ±0.12 log cfu/ml lactic acid bacteria

reductions) in the fresh squeezed white grape juice. The reason for this, more UV dosages was supplied in the low flow rate (19.30 mJ/cm^2) with more UV exposure times (24 min) different than high flow rate (8 mj/cm^2 and 10.26 min) (Table 4.7). However, temperature cannot controlled during the experiments for low and high flow rate. Temperature increased 8˚C in the low flow rate because of more waiting time in the system during cycling and the same raise (8˚C) were observed for the high flow rate owing to faster motion of the fluid in the system that caused heating of the juice.

On the other hand, medium flow rate provided the most effective microbial reductions as 1.70 ±0.028 log cfu/ml yeasts and 2.49 ±0.0031 log cfu/ml lactic acid bacteria at 19.96 mJ/cm^2 UV dosage. The reason of obtaining more UV dose in the medium flow rate is using grape juice which had better optical properties than the juice samples used for other treatments. Additionally, less temperature increase were recorded as 5.5 ˚C than other flow rates during medium flow rate experiment of the continuous flow UV study and this can be affect the microbial reduction.

Flow conditions are very important for the continuous flow UV studies. Keyser et al. (2008) were studied the turbulent flow conditions and more microbial reductions as 3.0 and 3.5 log cfu/ml were achieved for yeasts and aerobic bacteria in the apple juice at 234 mJ/cm^2 UV dosage because the juice were passed through the system as a thinner film (5 mm distance from the lamp). In our commercial system, the UV path length was 5.08 cm and optical properties of juice was different than their study. On the other hand, suspended particles found in fresh squezed grape juice adversely affected the UV inactivation process. That's why we obtained lower reductions in spoilage microorganisms found in fresh squeezed grape juice. In order to provide higher reductions, continuous flow UV reactor system should be designed to provide small UV path length distance for efficient UV inactivation of opaque juices. However, according to Koutchma et al. (2006), turbulent flow conditions were not very good for high turbid material as apple cider. They experienced laminar and turbulent flow conditions for the juice and the same decimal inactivation were obtained by laminar flow with using less UV dosages (25.1 to 18.8 mj/cm^2 at 56.8 ml/s flow rate) than turbulent flow conditions (90 and 150 mj/cm^2 at 1250 ml/s).

Table 4.9. Decimal reduction dose ($D_{(10)}$) and inactivation rate constants (k) of fresh squeezed white grape juice samples in the continuous flow UV reactor system.

Flow rates	**k (cm^2/mJ)**		**$D_{(10)}$ (mJ/cm^2)**		**R- squared value**	
	yeasts	**LAB**	**yeasts**	**LAB**	**yeasts**	**LAB**
Low flow (820 ml/min)	0.0899	0.1038	11.12	9.63	0.9815	0.9223
Medium flow (1516 ml/min)	0.0954	0.1422	10.48	7.03	0.9573	0.9901
High flow (2280 ml/min)	0.112	0.1767	8.93	5.66	0.9639	0.9621

LAB: lactic acid bacteria

$D_{(10)}$ and k values were calculated from the linear models in the logarithmic reduction versus UV dose graphs (Figure 4.27, 4.29 and 4.31). According to the Table 4.9, $D_{(10)}$ values of yeasts and lactic acid bacteria at the medium flow rate (10.48 mJ/cm^2 for yeasts and 7.03 mJ/cm^2 for lactic acid bacteria) were less than low flow rate (11.12 mJ/cm^2 for yeasts and 9.63 mJ/cm^2 lactic acid bacteria). $D_{(10)}$ and k values of high flow rate were not very reliable because there were some fluctuations about logarithmic reductions in the high flow rate. Linear curve was not initiated the first cycle (Figure 4.31). Although high r^2 values were obtained, models cannot reflect true information.

Tran and Farid (2004) reported that 87 and 119 mJ/cm^2 UV dosage was necessary for one log reduction of aerobic bacteria and yeasts and moulds in the freshly squeezed orange juice in a continuous flow UV reactor system with a 5 cm diameter. Their $D_{(10)}$ values were higher than our findings. The reason of this might be use of different juice samples having different optical properties. In their study, they used orange juice which is more turbid than white grape juice resulted in higher UV dosage values in their system.

Yeasts are more resistant microorganisms to the UV light than lactic acid bacteria and have lower k values. In all flow rates, k values of yeasts less than lactic acid bacteria in this study. For example, in the medium flow rate, k values of lactic acid bacteria were 0.1422 cm^2/mJ while it was lower as 0.0954 cm^2/mJ for yeasts (Table 4.9).

4.2.2.3. Effect of Continuous Flow UV Inactivation on the Physical, Chemical and Optical Properties of White Grape Juice Samples

It follows from this study, UV irradiation can be used as a disinfection method for the inactivation of white grape juice. UV inactivation method cannot be only evaluated according to microbiological aspects, it should also maintain quality parameters of the juice product without any change such as off-flavor, off-odor or discoloration. For that purpose, some physical, chemical and optical parameters of the pasteurized and fresh squeezed white grape juice samples were estimated in terms of before and after UV irradiation results (Table 4.10 and 4.11).

Two sample t-test were applied to the data in order to measure change of quality paramaters. According to the table 4.10 and 4.11, pH of the juice were not affected by the UV irradiation. Noci et al. (2008) were also found that pH was not the noticeable factor in the UV disinfection of the apple juice. Turbidity values of the pasteurized white grape juice were decreased after UV treatment at low and medium flow rate because microorganisms were inactivated and juice turned into less cloudy. At the high flow rates turbidity wasnot different because of very low microbial inactivation rate. Turbidity of the freshly squeezed white grape juice was not changed due to presence of suspended particles. Literatures were reported that brix values were not affected by the UV irradiation (Noci, et al. 2008). Although total soluble solids (brix) of freshly squeezed white grape juice were not altered, the brix of pasteurized samples was raised in this study. Similarly, densities of the pasteurized white grape juice were increased depending on increasing brix values.

One of the most important factors was the absorbance value of the samples. Reduction of absorbance coefficients were higher in freshly squeezed white grape juice compared to pasteurized juice. These values were (13.25, 13.9 and 15.13 cm^{-1}) before UV, although lower data were obtained (7.63, 6.29, 10.23 cm^{-1}) for three flow rates. The reason is that L-ascorbic acid added in the fresh squeezed juice was degraded by UV irradiation and caused lower absorption coefficient of the juice (Koutchma, et al. 2009). Tran and Farid (2004) also observed 17% vitamin C degradation after 100 mJ/cm^2 UV treatment. Titratable acidity of the juice samples may be also decreased depending on reduction of the ascorbic acid amount by UV light and reduction of volatile acids by the effect of temperature increase.

Colour parameters were varied slightly after the continuous flow UV treatment. Although less changes were observed in L*(lightness) values, a* (redness) and b* (greenness) were changed highly after UV irradiation(Table 4.11). The same results were obtained for fresh squeezed apple juice (Noci, et al. 2008) Browning index (BI) was not different in the pasteurized juice however more brown color was gained after UV irradiation of fresh squeezed juice. The reason is that enzymes cannot be inactivated by irradiation in the fresh squeezed juice. Additionally, L-ascorbic acids in the fruit juice cause decrease in L* value (more darker juice) due to browning.

Table 4.10. Physical, chemical, and optical properties of pasteurized white grape juice samples before and after continuous flow UV irradiation.

Pasteurized white grape juice						
FLOW RATES	**Before UV**			**After UV**		
	low	**medium**	**high**	**low**	**medium**	**high**
UV dosages						
pH	3.45 ±0.007	3.44 ±0.007	3.44 ±0.007	3.46 ±0.007	3.42 ±0.007	3.45 ±0.007
temperature (C)	30.4	31	28.5	35.8	34.8	33.4
turbidity (NTU)	32.5 ±0.14	26.6 ±01.13	6.74 ±0,06	*7.7 ±0.03	*7.87 ±0.26	*7.36 ±0.13
brix (%)	17.42 ±0.007	16.84 ±0.01	17.49 ±0.007	*17.88 ±0.007	*18.13 ±0.00	*17.83 ±0.001
density (g/cm3)	1.0698	1.0651	1.0701	*1.072	*1.0714	*1.0716
abs coef.(at 254nm)	5.63 ±0.007	5.19 ±0.00	5.64 ±0.007	*4.85 ±0.007	*4.65 ±0.00	*4.91 ±0.007
colour (ΔE)	51.5	52.13	51.41	52.41	52.62	52.43
(BI)	57.65 ±0.05	57.34 ±0.05	57.52 ±0.02	57.51 ±0.003	57.38 ±0.02	57.54 ±0.02
L*	51.45 ±0.023	52.09 ±0.22	51.36 ±0.18	*52.36 ±0.169	52.58 ±0.14	*52.38 ±0.15
a*	1.30 ±0.007	1.30 ±0.014	1.27 ±0.04	*1.10 ±0.011	*1.09 ±0.03	1.17 ±0.04
b*	2.05 ±0.09	1.58 ±0.07	1.86 ±0.007	2.01 ±0.005	1.82 ±0.02	2.02 ±0.005
titratable acidity (%)	0.372 ±0.001	0.387 ±0.00	0.383 ±0.008	0.368 ±0.001	*0.371 ±0.001	*0.378 ±0.003

*: Significant (p value <0.05)

Table 4.11. Physical, chemical, and optical properties of fresh squeezed white grape juice samples before and after continuous flow UV irradiation.

Fresh squeezed white grape juice						
FLOW RATES		**Before UV**			**After UV**	
		Low	**medium**	**high**	**low**	**medium**	**high**
UV dosages							
pH		4.40 ±0.007	4.33 ±0.035	4.32 ±0.007	4.42 ±0.007	4.34 ±0.02	4.34 ±0.01
temperature (˚C)		25	21.5	26	33	27	34
turbidity (NTU)		105 ±2.12	80.1 ±0.64	113 ±0.7	107 ±0.7	*71.6 ±0.14	*109 ±0.00
brix (%)		16.38 ±0.00	15.74 ±0.007	16.98 ±0.007	16.45 ±0.02	15.75 ±0.014	17.16 ±0.007
density (g/cm3)		1.0643	1.0641	1.067	1.0648	1.0641	1.0677
abs coef.(254nm)		13.25	13.9	15.13	*7.63	*6.59	*10.23
colour	**(ΔE)**	53.3	52.86	52.09	51.78	52.64	52.30
	(BI)	58.36 ±0.02	58.19 ±0.008	56.86 ±0.015	*58.63 ±0.001	*59.12 ±0.02	*57.00 ±0.01
	L*	53.30 ±0.21	52.72 ±0.05	52.07 ±0.005	*51.60 ±0.15	52.41 ±0.035	52.28 ±0.19
	a*	-0.03 ±0.03	-0.03 ±0.007	1.10 ±0.02	*0.24 ±0.014	*0.64 ±0.00	1.11 ±0.03
	b*	4.29 ±0.02	3.95 ±0.014	0.91 ±0.01	*4.39 ±0.007	*4.96 ±0.028	1.14 ±0.03
Titratable acidity (%)		0.174 ±0.001	0.234 ±0.008	0.179 ±0.001	*0.131 ±0.001	*0.133 ±0.003	*0.150 ±0.001

*: Significant (p value <0.05)

4.2.3. Shelf Life Results

Microbiological shelf life results during 13 days for untreated and irradiated white grape juice was shown in Table 4.12. According to the logarithmic data, untreated fresh squeezed white grape juice was spoiled within 10 days at refrigerator because the juice reached to aproximately 5000 cfu/ml (3.7 log cfu/ml) (Tran and Farid, 2004) after 10 days. However, juice treated with 126 mJ/cm^2 UV dose didnot spoiled and contained less microbial load than 5000 cfu/ml reported by Tran and Farid (2004) as 1.53 log

cfu/ml (33.88 cfu/ml) total count, 0.79 log cfu/ml yeasts (6.17 cfu/ml) and 1.06 log cfu/ml (11.48 cfu/ml) lactic acid bacteria after 13 days.

Table 4.12. Microbiological results of fresh squeezed white grape juice during 13 days.

days	**Untreated**			**UV-treated**		
	total counts	**yeasts**	**lactic acid bacteria**	**total counts**	**yeasts**	**lactic acid bacteria**
0	2.14±0.197	0.00	1.81±0.047	0.75±0.18	0.33±0.23	0.00
1	1.81±0.047	0.00	1.57±0.38	0.73±0.51	0.00	0.00
3	2.15	0.5±0.7	1.60	1.25±0.18	0.58±0.05	0.25±0.18
6	2.32±0.06	1.75±0.2	2.05±0.13	1.5±0.28	0.25±0.17	0.33±0.23
10	3.83±0.07	3.57±0.1	3.7±0.16	0.5	0.40±0.28	0.33±0.23
13	5.37±0.001	5.17±0.09	5.06±0.035	1.53±0.09	0.79±0.55	1.06±0.4

CHAPTER 5

CONCLUSION

Pasteurized white grape juice inoculated with *S. cerevisiae* (Y139) and naturally spoiled fresh squeezed white grape juice were disinfected by using static bench top collimated beam apparatus and contiunous flow UV reactor system. Before UV irradiation treatments, physical, chemical and optical parameters of the white grape juice were measured and rheological parameters were determined. Results of rheological studies indicated that pasteurized and fresh squeezed white grape juice were newtonian fluids, because their viscosities were not time dependent. Viscosities of the white grape juice samples were constant by increasing time at a constant shear rate (100 rpm). However, raising temperature of the juice from refrigerator temperature (0˚C) to pasteurization temperature (90˚C), cause a decreas in viscosities. Density behaviour of the pasteurized and fresh squeezed white grape juice were analysed for the same temperature range. In addition to these rheological experiments, particle size distribution of the freshly squeezed white grape juice were measured to learn the effect of suspended particles on the UV inactivation of microorganisms.

Bench top UV irradiation treatment was performed in order to derive UV dose curve for inactivation of the microorganisms in the white grape juice samples. *S. cerevisiae* (Y139) strains with two different inoculation level (5.50 ±0.107 log cfu/ml and 7.33 ±0.013 log cfu/ml) were completely inactivated within 6 min at the UV intensity of Io= 0.91 mW/cm^2 (228.96 mJ/cm^2 UV dosage). However, at the low UV intensity (Io= 0.36 mW/cm2), more UV exposure times (9 min and 12 min) and UV doses (136.08 mJ/cm^2 and 181.44 mJ/cm^2) were required for the samples having 5.50 ±0.107 log cfu/ml and 7.33 ±0.013 log cfu/ml initial load. Natural spoilage microflora in the fresh squeezed juice were identified as yeasts and lactic acid bacteria. After bench top UV irradiation, the yeast was decreased by 2.41 log and lactic acid bacteria was reduced by 3.13 log in the fresh squeezed juice at the UV intensity of Io= 0.76 mW/cm^2 (105.89 mJ/cm^2 UV dosage).

The pasteurized white grape juice inoculated with *S. cerevisiae* (Y139) and fresh squeezed white grape juice with spoilage microflora (yeasts and lactic acid bacteria)

were passed throughout the system eight times at three different level of flow rates i.e. low, medium and high flow rate in this study. The highest microbial reduction of *S. cerevisiae* were achieved as 3.39 ±0.044 log cfu/ml at the low flow rate (820 ml/min). Because, the low flow rate had the highest UV dosages (56.06 mJ/cm^2) providing the longest exposure time (24.27 minutes). Additionally, there were high correlations between logaritmic reduction rate (log N/No) and UV dosages.

After cycling of fresh squeezed white grape juice eight times, the most effective microbial reductions were detected at the medium flow rate (1516 ml/min). 1.70 ±0.028 log cfu/ml reduction was obtained for the yeast counts and 2.49 ±0.0031 log cfu/ml reduction was observed for lactic acid bacteria count at UV dosages of 19.96 mJ/cm^2. The main reason for this is the usage of grape juice which has better optical quality than the juice used in other treatment conditions. Temperature increase may be another reason. Although temperature was increased as 8 ˚C at high (2085 ml/min) and low flow rates (774 ml/min), medium flow rates enhanced temperature only 5 ˚C. Therefore, temperature seems to be an important factor on the inactivation study of the microorganisms and should be controlled during treatment.

Some physical, chemical and optical properties of the white grape juice samples were measured before and after continuous flow UV reactor irradiation in order to evaluate the effect ot UV irradiation on the quality parameters of the juice. pH was not effected. Turbidity values of pasteurized white grape juice decreased because of microbial inactivation. However, turbidity of the fresh squeezed white grape juice was not different because of its highly turbid structure. The raise in brix and density values of the pasteurized juice may be explained by the temperature effect. Similarly, decrease in the titratable acidity can also be due to evaporating of volatile acids and degradation of ascorbic acid. Absorbance of the freshly squeezed white grape juice were greatly affected because of ascorbic acid degradation by the UV irradiation. Color was significanlty change and darker color was obtained from the freshly squeezed white grape juice with less L* value because of inefficient inactivation of enzymes.

Fresh squeezed white grape juice exposured to UV light with a constant intensity of Io= 0.76 mW/cm^2 stored at refrigerator temperature (4 ˚C). Microbial growth of control (untreated) and UV treated juice were counted for total count, yeasts and lactic acid bacteria for every 2 or 3 days. After 10 days, untreated white grape juice

were reached to the spoilage count that was 3.7 log cfu/ml (5000 cfu/ml) although it was only 1.53 log cfu/ml (33.88 cfu/ml) for UV treated juice.

In future, more efficient continuous flow UV reactor systems should be designed (with less path length) in terms of juice characteristics and temperature must be controlled during the experiments.

REFERENCES

Aguilar-Rosas, S.F., Ballinas-Casarrubias, M.L., Nevarez-Moorillon, G.V., Martin-Belloso, O., Ortega-Rivas, E. 2007. Thermal and pulsed electric fields pasteurization of apple juice: Effects on physicochemical properties and flavour compounds. *Journal of Food Engineering*, 83, 41-46.

Aguilo´-Aguayo, I., Soliva-Fortuny, R., Martı´n-Belloso, O. 2010. Impact of high-intensity pulsed electric field variables affecting peroxidase and lipoxygenase activities of watermelon juice. *LWT - Food Science and Technology*, 43, 897–90.

Anlı, R.E. 2006. Sofralık üzüm. *Bağlar güzeli, üzüm ve üzüm kültürü*, 61-88.

Aqua Best. 2007. The electromagnetic spectrum. http://www.aquabest.net/UV.asp?id=305 (October 2011).

Artes, F., Allende, A. 2005. Minimal Fresh Processing of Vegetables, Fruits and Juices. *Emerging technologies for food processing*, 26, 677-716.

Artes, F., Allende, A.. 2005. Minimal Fresh Processing of Vegetables, Fruits and Juices. *Emerging technologies for food processing*, 26, 676-757.

Artık, N., Velioğlu, S. 1992. Meyve suyunun kimyasal bileşimi, işletme ve depolama sırasında değişmesi. *Meyve suyu endüstrisinde kalite kontrol semineri*, Ankara Üniversitesi Ziraat Fakültesi Gıda Bilimi ve Teknolojisi Bölümü, 9-13 Mart, Ankara.

Ashurts, P.R. 2005. *Chemistry and technology of soft drinks and fruit juices* (Second edition), Blackwell Publishing .

Atılgan, M.R. 2007. Disinfection of Liquid Egg Products by Using UV Light. MS Thesis. Izmir Institute of Technology.

Barbosa-Canovas, G.V., Tapia, M.S., Cano, M.P. 2005. *Novel food processing Technologies.* Marcel Dekker, New York.

Barker, B. 2008. Massecuite consistency measurement using a pipeline viscometer. *Proc S Afr Sug Technol Ass*, 81, 227-233.

Bates, R.P., Morris, J.R. and Crandall, P.G. 2001. Principles and Practices of Small and Medium-Scale Fruit Juice Processing. *FOA Agricultural Services Bulletin*, Food Science and Human Nutrition Department University of Florida, United States, 146.

Begum, M., Hocking, A.D., Miskelly, D. 2009. Inactivation of food spoilage fungi by ultraviolet (UVC) irradiation. *International Journal of Food Microbiology*, 129, 74–77.

Beltrán, J.G. and Barbosa-Cánovas G.V. 2005. Reduction of *Saccharomyces cerevisiae*, *Escherichia coli* and *Listeria innocua* in apple juice by ultraviolet light. *Journal of food process engineering*, 28, 437-452.

Betoret, E., Betoret, N., Carbonell, J.V., Fito, P. 2009. Effects of pressure homogenization on particle size and the functional properties of citrus juices. *Journal of Food Engineering*, 92, 18-23.

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R.K. 2000. Existing and potential applications of ultraviolet light in the food industry – a critical review. *Journal of the Science of Food and Agriculture*, 80, 637- 645.

Borbalan, A.M.A., Zorro, L., Guille´n, D.A., Barroso, C.G. 2003. Study of the polyphenol content of red and white grape varieties by liquid chromatography–mass spectrometry and its relationship to antioxidant power. *Journal of Chromatography A.*, 1012, 31-38.

Burdurlu, H.S., Karadeniz, F. 2003. Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry*, 80, 91-97.

Butz, P., Tauscher , B. 2002. Emerging technologies: chemical aspects. *Food Research International* 35, 279-284.

Cantwell, R.E., Hofmann, R. 2008. Inactivation of indigenous coliform bacteria in unfiltered surface water by ultraviolet light. *Water research*, 42, 2729 -2735.

Cemeroğlu, B., Yemenicioğlu, A., Özkan, M. 2001. Meyve ve sebzelerin bileşimi soğukta depolanmaları. *Meyve sebze işleme teknolojisi*, Ankara.

Cemeroğlu, B. 2004. Meyve ve Sebze İşleme Teknolojisi. Ankara: Başkent Klişe Matbaacılık.

Char, C.D., Mitilinaki, E., Guerrero, S.N., Alzamora, S.M. 2010. Use of High-Intensity Ultrasound and UV-C Light to Inactivate Some Microorganisms in Fruit Juices. *Food Bioprocess Technol.*, 3(6), 797-803.

Charles-Rodrı´guez, A.V., Neva´rez-Moorillo´n, G.V., Zhang, Q.H., Ortega-Rivas, E. 2007. Comparison of thermal processing and pulsed electric fields treatment in pasteurization of apple juice. *Food and Bioproducts Processing*, 85(C2), 93-97.

Chick, H. 1908. An investigation into the laws of disinfection. *Journal of Hygiene* (Cambridge), 8, 92-158.

Chin, N.L., Chan, S.M., Yusof, Y.A., Chuah, T.G., Talib, R.A. 2009. Modelling of rheological behaviour of pummelo juice concentrates using master-curve. *Journal of Food Engineering*, 93, 134-140.

Choi, Y., Choi, Y.J. 2010. The effects of UV disinfection on drinking water quality in distribution systems. *Water research*, 44, 115-122.

Chun, H., Kim, J., Chung, K., Won, M., Song, K.B. 2009. Inactivation kinetics of Listeria monocytogenes, *Salmonella enterica serovar Typhimurium, and Campylobacter jejuni* in ready-to-eat sliced ham using UV-C irradiation. *Meat Science*, 83, 599-603.

Cook, K.A., Dobbs, T.E., Hlady, W.G., Wells, J.G., Barrett, T.J., Puhr, N.D., Lancette, Cortés, C., Esteve, M.J., Rodrigo, D., Torregrosa, F., Frı´gola, A. 2006. Changes of colour and carotenoids contents during high intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology*, 44, 1932-1939.

Cosme, F., Ricardo-Da-Silva, J.M., Laureano, O. 2009. Tannin profiles of Vitis vinifera L. cv. red grapes growing in Lisbon and from their monovarietal wines. *Food Chemistry*, 112, 197-204.

Cruess, W.V. 1938. *Commercial fruit and vegetable products* (Second edition), 292-298.

Cunha, S.C., Fernandes, J.O., Faria, M.A., Ferreira, I. M. P. V. L. O., Ferreiara, M.A. 2002. Quantification of organic acids in grape musts and port wines. *Cienc. Tecnol. Aliment.*, 3(4), 212-216.

Dani, C., Oliboni, L.S., Vanderlinde, R., Bonatto, D., Salvador, M., Henriques, J.A.P. 2007. Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. *Food and Chemical Toxicology*, 45, 2574-2580.

Elez-Martınez, P., Escola-Hernandez, J., Soliva-Fortuny, R.C., Martin-Belloso, O. 2005. Inactivation of Lactobacillus brevis in orange juice by high-intensity pulsed electric fields. *Food Microbiology*, 22, 311–319.

Falguera, V., Pagán, J., Garza, S., Garvín, A., Ibarz, A. 2011. Ultraviolet processing of liquid food: A review. Part 1: Fundamental engineering aspects. *Food Research International*, 44, 1571-1579.

Federal Register [FR]. 2001. Hazard analysis and critical control points (HACCP); procedures for the safe and sanitary processing and importing of juice. U.S. Food and Drug Administration. Final rule. *Federal Register*, 66, 6138–6202.

Feng, M., Mei, J., Hu, S., Janney, S., Carruthers, J., Holbein, B., Huber, A., Kidby, D. 1997. Selective removal of iron from grape juice using an iron (III) chelating resin. *Separation and Purification Technology*, 11, 127-135.

Fonseca, J.M., Rushing, J.W. 2006. Effect of ultraviolet-C light on quality and microbial population of fresh-cut watermelon. *Postharvest Biology and Technology* , 40, 256–261

Fratianni, A., Cinquanta, L., Panfili, G. 2010. Degradation of carotenoids in orange juice during microwave heating. *LWT - Food Science and Technology*, xxx, 1-5.

Fremont, L. 2000. Minireview biological effects of resveratrol. *Life Sciences*, 66(8), 663-673.

G.A., Bodager, D.W., Toth, B.L., Genese, C.A., Highsmith, A.K., Pilot, K.E., Finelli, L. and Swerdlow, D.L. 1998. Outbreak of Salmonella Serotype Hartford Infections Associated With Unpasteurized Orange Juice. *JAMA*, 280(17), 1504-1509.

Gabriel, A.A., Nakano, H. 2009. Inactivation of Salmonella, E. coli and Listeria monocytogenes in phosphate-buffered saline and apple juice by ultraviolet and heat treatments. *Food control*, 20, 443-446.

Garde-Cerda´n, T., Arias-Gil, M., Marselle´s-Fontanet, A.R., Ancı´n-Azpilicueta, C., Martı´n-Belloso, O. 2007. Effects of thermal and non-thermal processing treatments on fatty acids and free amino acids of grape juice. *Food Control*, 18, 473–479.

Gardner, P.T., White, T.A.C., McPhail, D.B., Duthie, G.G. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*, 68, 471-474.

Gómez, P.L., Alzamora, S.M., Castro, M.A., Salvatori, D.M. 2010. Effect of ultraviolet-C light dose on quality of cut-apple: Microorganism, color and compression behavior. *Journal of Food Engineering*, 98, 60–70.

Gonzalez-Barrio, R., Vidal-Guevara, M.L., Tomás-Barberán, F.A., Espín, J.C. 2009. Preparation of a resveratrol-enriched grape juice based on ultraviolet C-treated berries. *Innovative Food Science and Emerging Technologies*, 10, 374-382.

Green, S., Basaran, N., Swanson, B.G. 2003. High İntensity Light. *Food preservation techniques*, Ed. by Peter Zeuthen and Leif Bùgh-Sùrensen, CRC press, 284-301.

Hakgüder, B. 2009. UV Disinfection of Some of the Fruit Juices. MS Thesis. Izmir Institute of Technology.

Healthy Menu Mailer. 2005-2007. Benefits Of Grape Juice-Battle Fatigue And Keep Your Heart Healthy. http://www.healthymenumailer.com/articles/benefits-grape-juice.html (October, 2011).

Heraeus noblelight. 2011. Ultraviolet Light for Industrial Food Processing Disinfection and Oxidation. http://heraeus-noblelight.com/media/webmedia_local/media/pdf/uv/b118_e_ultravioletlightforindustrialfoodprocessing.pdf (November, 2011).

Hijnen, W.A.M., Beerendonk, E.F., Medema, G.J. 2010. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water research*, 40, 3-22.

Ibarz, A., Pagan, J., Panades, R., Garza, S. 2005. Photochemical destruction of color compounds in fruit juices. *Journal of Food Engineering*, 69, 155-160.

Jacobs, M.B., Ph.D. 1951. *The Chemistry and Technology of Food and Food Products* (Second edition), 3, 2200-2201.

Johnson, K.M., Anil Kumar, M.R., Ponmurugan, P., Gananamangai, M.B. 2010. Ultraviolet radiation and its germicidal effect in drinking water purification *Journal of Phytology*, 2(5), 12-19.

Karel, M., Lund, D.B. 2003. *Physical principles of food preservation* (Second revised edition), Marcel Dekker, New York.

Keyser, M., Műller, I.A., Cilliers, F.P., Nel, W., Gouws, P.A. 2008. Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Science and Emerging Technologies*, 9, 348–354.

Kouchma, T.N., Forney, L.J., Moraru, C.I. 2009. Ultraviolet light in food technology, principles and applications.

Koutchma, T. 2008. UV Light for Processing Foods. *Ozone: Science and Engineering*, 30, 93-98.

Koutchma, T., Keller, S., Chirtelc, S., Parisi, B. 2008. Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. *Innovative Food Science and Emerging Technologies*, 5, 179–189.

Koutchma, T., Parisi, B. and Patazca, E. 2007. Validation of UV coiled tube reactor for fresh juices. *Journal of Environmental Engineering Science*, 6, 319-328.

Koutchma, T., Parisi, B., Unluturk, S.K. 2006. Evaluation of UV Dose in Flow-Through Reactors for Fresh Apple Juice and Cider. *Chem. Eng. Comm.*, 193, 715-728.

Kowalski, W. 2009. Ultraviolet germicidal irradiation handbook. *UVGI air and surface disinfection*, Springer-Verlag Berlin Heidelberg.

Igual, M., García-Martínez, E., Camacho, M.M., Martínez-Navarrete, N. 2010. Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. *Food Chemistry*, 118, 291–299.

Manzocco, L., Pieve, S.D., Maifreni, M. 2011. Impact of UV-C light on safety and quality of fresh-cut melon. *Innovative Food Science and Emerging Technologies*, 12, 13-17.

Marselles-Fontanet, A.R., Martin-Belloso, O. 2007. Optimization and validation of PEFprocessing conditions to inactivate oxidative enzymes of grape juice. *Journal of food engineering*, 83, 452-462.

MEYED- Meyed Meyve Suyu Endüstrisi Derneği, İstatiki Değerlendirme (2000-2008). 2008. http://www.meyed.org.tr/content/files/istatistikler/2008.pdf (October, 2011).

Murakami, E.G., Jackson, L., Madsen, K. and Schickedanz, B. 2006. Factors affecting the ultraviolet inactivation of Escherichia coli K12 in apple juice and a model system. *Journal of food process engineering*, 29, 53-71.

Ngadi, M., Smith, J.P., and Cayouette, B. 2003. Kinetics of ultraviolet light inactivation of *Escherichia coli* O157:H7 in liquid foods. *Journal of the Science of Food and Agriculture*, 83, 1551-1555.

Nindo, C.I., Tang, J., Powers, J.R., Singh, P. 2005. Viscosity of blueberry and raspberry juices for processing applications. *Journal of Food Engineering*, 69, 343–350.

Noci, F., Riener, J., Walkling-Ribeiro, M., Cronin, D.A., Morgan, D.J., Lyng, J.G. 2008. Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple Juice. *Journal of food engineering*, 85, 141-146.

Organic facts. 2011. Health benefits of grapes. http://www.organicfacts.net/health-benefits/fruit/health-benefits-of-grapes.html (September, 2011).

Pathanibul, P., Taylor, T.M., Davidson, P.M, Harte, F. 2009. Inactivation of *Escherichia coli* and *Listeria innocua* in apple and carrot juices using high pressure homogenization and nisin. *International Journal of Food Microbiology* 129, 316–320.

Pederson, C.S. 1980. Grape juice. *Fruit and vegetables juice processing technology*, Nelson and Tressler (eds.), AVI Publishing Co., Westport. CT, 268-309.

Peña, M.M., Salvia-Trujillo, L., Rojas-Graü, M.A., Martín-Belloso. O. 2011. Changes on phenolic and carotenoid composition of high intensity pulsed electric field and thermally treated fruit juice–soymilk beverages during refrigerated storage. *Food Chemistry*, 129, 982-990.

Pereira, R.N., Vicente, A.A. 2010. Environmental impact of novel thermal and non-thermal technologies in food processing. *Food Research International*, 43, 1936-1943.

Perman, J.A. 1996. Digestion and absorption of fruit juice carbohydrates. *Journal of the American College of Nutrition*, 15(5), 12S-17S.

Rai, P., De, S. 2009. Clarification of pectin-containing juice using ultrafiltration. *Current science*, 96(10), 1361-1371.

Rajashekhara, E., Suresh, E.R., Ethiraj, S. 2000. Modulation of thermal resistance of ascospores of Neosartorya ¢scheri by acidulants and preservatives in mango and grape juice. *Food Microbiology*, 17, 269-275.

Raso, J. and Barbosa-Cánovas, G.V. 2003. Nonthermal Preservation of Foods Using Combined Processing Techniques. *Critical Reviews in Food Science and Nutrition*, 43(3), 265–285.

Ray, B. 2004. *Fundemantal food microbiology* (Third edition), CRC Press: Baco Raton, Florida, 279-280; 455-465.

Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Publication. 2006. Meyve suyu üretim teknolojisi. *Gıda Serisi, 2, Ankara.*

Riener, J., Noci, F., Cronin, D.A., Morgan, D.J., Glyng, J.G. 2008. Combined effect of temperature and pulsed electric fields on apple juice peroxidase and polyphenoloxidase inactivation. *J.G., Food Chemistry*, 109, 402-407.

Rivas, A., Rodrigo, D., Martinez, A., Barbosa-Cánovas, G.V., Rodrigo, M. 2006. Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. *LWT*, 39, 1163-1170.

Romo, L.A.R. 2004. Control of *Salmonella enterica* serovar enteritidis in shell eggs by ozone, ultraviolet radiation, and heat. Ph.D. Dissertation, The Ohio State University.

Salvia-Trujillo, L., Peña, M.M., Rojas-Graü, M.A., Martín-Belloso, O. 2011. Microbial and enzymatic stability of fruit juice-milk beverages treated by high intensity pulsed electric fields or heat during refrigerated storage. *Food Control*, 22, 1639-1646.

Schenk,M., Raffellini, S., Guerrero, S., Blanco,G.A., Alzamora, S.M. 2011. Inactivation of Escherichia coli, Listeria innocua and Saccharomyces cerevisiae by UV-C light: Study of cell injury by flow cytometry. *LWT-Food science and technology*, 44, 191-198.

Schilling, S., Schmid, S., Jager, H., Ludwig, M., Dietrich, H., Toepfl, S., Knorr, D., Neidhart, S., Schieber, A., Carle, R. 2008. Comparative Study of Pulsed Electric Field and Thermal Processing of Apple Juice with Particular Consideration of Juice Quality and Enzyme Deactivation. *J. Agric. Food Chem.*, 56, 4545-4554.

Somogyi, L.P., Barrett, D.M., Hui, Y.H. 1996. Major processed products. *Processing fruits : Science and Technology*, 2, 197-217.

Souci, S.W., fachmann, W., kraut, H. 1989-1990. Juice from fruits and berries. *Food composition and nutrition tables* (Fourth Edition), 915-916.

Soyer, Y., Koca, N., Karadeniz, F. 2003. Organic acid profile of Turkish white grapes and grape juices. *Journal of Food Composition and Analysis* 16, 629-636.

Talcott, S.T., Brenes, C.H., Pires, D.M., Pozo-Insfran D.D. 2003. Phytochemical Stability and Color Retention of Copigmented and Processed Muscadine Grape Juice. *Journal of Agricultural and Food Chemistry*, 51, 957-963.

The Perfect Berry. 2007. The Health Benefits of White Grapes. http://www.theperfectberry.com/health_benefits_of_white_grapes.html (January, 2009).

Timmermans, R.A.H., Mastwijk, H.C., Knol, J.J., Quataert, M.C.J., Vervoort, L., Van der Plancken, I., Hendrickx, M.E., Matser , A.M. 2011. Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I: Impact on overall quality attributes. *Innovative Food Science and Emerging Technologies*, 12, 235–243.

Ting, S.V., Deszyck, E.J. 1960. Total amino acid content of chilled orange juice and frozen orange concentrate. *Florida Agricultural Experiment Stations Journal Series*, 1184, 252-257.

Tiwari, B.K, Muthukumarappan, K., O'Donnell, C.P., Cullen, P.J. 2008. Effects of Sonication on the Kinetics of Orange Juice Quality Parameters. *Journal of Agricultural and Food Chemistry* 56, 2423-2428.

Tiwari, B.K., Muthukumarappan, K., O'Donnell, C.P. and Cullen, P.J. 2008. Modelling color degradation of orange juice by ozone treatment using response surface methodology. *Journal of Food Engineering*, 88, 553-560.

Tiwari, B.K., O'Donnella, C.P., Cullen, P.J. 2009. Effect of non thermal processing technologies on the anthocyanin content of fruit juices. *Trends in Food Science & Technology*, 20, 137-145.

Tran, M.T.T., Farid, M. 2004. Ultraviolet treatment of orange juice. *Innovative food science and emerging Technologies*, 5, 495-502.

Tournasa, V.H., Heeres, J., Burgess, L. 2006. Moulds and yeasts in fruit salads and fruit juices. *Food Microbiology*, 23, 684-688.

U.S. Food and Drug Administration. 2000. 21 CFR Part 179: Irradiation in the production, processing and handling of food. *Federal Register*, 65, 71056-71058.

Unluturk, S., Atılgan, M.R., Baysal, A.H., Tarı C. 2007. Use of UV-C radiation as a non-thermal process for liquid egg products (LEP). *Journal of food engineering*, 85, 561-568.

Unluturk, S., Atılgan, M.R., Baysal, A.H., Unluturk M.S. 2010. Modeling inactivation kinetics of liquid egg white exposed to UV-C irradiation. *International journal of food microbiology*, 142, 341-347.

USFDA. 2001. Hazard Analysis and Critical Control Point (HACCP): Procedures for the Safe and Sanitary Processing and Importing of Juice-Final Rule. *Federal Register*, U.S. Food and Drug Administration, Washington, DC, 66, 13.

Valappil, Z.A., Fan, X., Zhang, H.Q. and Rouseff, R.L. 2009. Impact of Thermal and Nonthermal Processing Technologies on Unfermented Apple Cider Aroma Volatiles. *J. Agric. Food Chem.***,** 57, 924-929.

Vantarakis, A., Affifi, M., Kokkinos, P., Tsibouxi, M., Papapetropoulou, M. 2011. Occurrence of microorganisms of public health and spoilage significance in fruit juices sold in retail markets in Greece. *Anaerobe*, xxx, 1-4.

Vojdani, J.D., Beuchat, L.R., Tauxe, R.V. 2008. Juice-Associated Outbreaks of Human Illness in the United States, 1995 through 2005. *Journal of Food Protection*, February, 71(2), 356-364.

Walkling-Ribeiro, M., Noci, F., Cronin, D.A., Riener,J., Lyng, J.G., Morgan, D.J. 2008. Reduction of Staphylococcus aureus and quality changes in apple juice processed by ultraviolet irradiation, pre-heating and pulsed electric fields. *Journal of food engineering*, 89, 267-273.

Welch's International. 2008. Health Benefits of White Grape Juice. http://www.welchsinternational.com/health/white.shtml (October, 2011).

Wicker, L., Ackerley, J.L., Hunter, J.L. 2003. Modification of pectin by pectinmethylesterase and the role in stability of juice beverages. *Food Hydrocolloids*, 17, 809–814.

Winiarska-Mieczan, A., Nowak, K. 2008. Determining the content of some minerals in fruit and vegetable baby juice. *J. Elementol.*, 13(3), 433-442.

World Health Organization and Food and Agriculture Organization of the United Nations. 1981. Codex standard for grape juice preserved exclusively by physical means (world-wide standard). *Codex alimentarius*, Codex Stan 82-1981.

World Health Organization and Food and Agriculture Organization of the United Nations. 2005. Codex general standard for fruit juices and nectars. *Codex alimentarius*, **C**odex Stan 247-2005.

Wu, Y., Mittal, G.S., Griffiths, M.W. 2005. Effect of Pulsed Electric Field on the Inactivation of Microorganisms in Grape Juices with and without Antimicrobials. *Biosystems Engineering*, 90(1), 1-7.

Wyckomar UV Purification Systems. 2011. Ultraviolet Sterilization Technology. http://www.wyckomaruv.com/PDFs/Ultraviolet_SterilizationTechnology.pdf (November, 2011).

Zhang, C., Trierweiler, B., Li, W., Butz, P., Xu, Y., Rüfer, C.E., Ma, Y., Zhao, X. 2011. Comparison of thermal, ultraviolet-c, and high pressure treatments on quality parameters of watermelon juice. *Food Chemistry*, 126, 254-260.

Zoecklein, B. 1991. Protein stability determination in juice and wine. *Viticulture/ enology*, Virginia cooperative extension publication, 463-015.

Zulueta, A., Esteve, M.J., Frasquet, I., Frigola, A. 2007. Vitamin C, vitamin A, phenolic compounds and total antioxidant capacity of new fruit juice and skim milk mixture beverages marketed in Spain. *Food Chemistry*, 103, 1365-1374.

Zuritz, C.A., Munoz Puntes, E., Mathey, H.H., Pe´rez, E.H., Gasco´n, A., Rubio, L.A., Carullo, C.A., Chernikoff, R.E., Cabeza, M.S. 2005. Density, viscosity and coefficient of thermal expansion of clear grape juice at different soluble solid concentrations and temperatures. *Journal of Food Engineering*, 7, 143–149.

APPENDIX A

MICROSCOBIC APPEARANCE OF NATURAL FLORA IN WHITE GRAPE JUICE

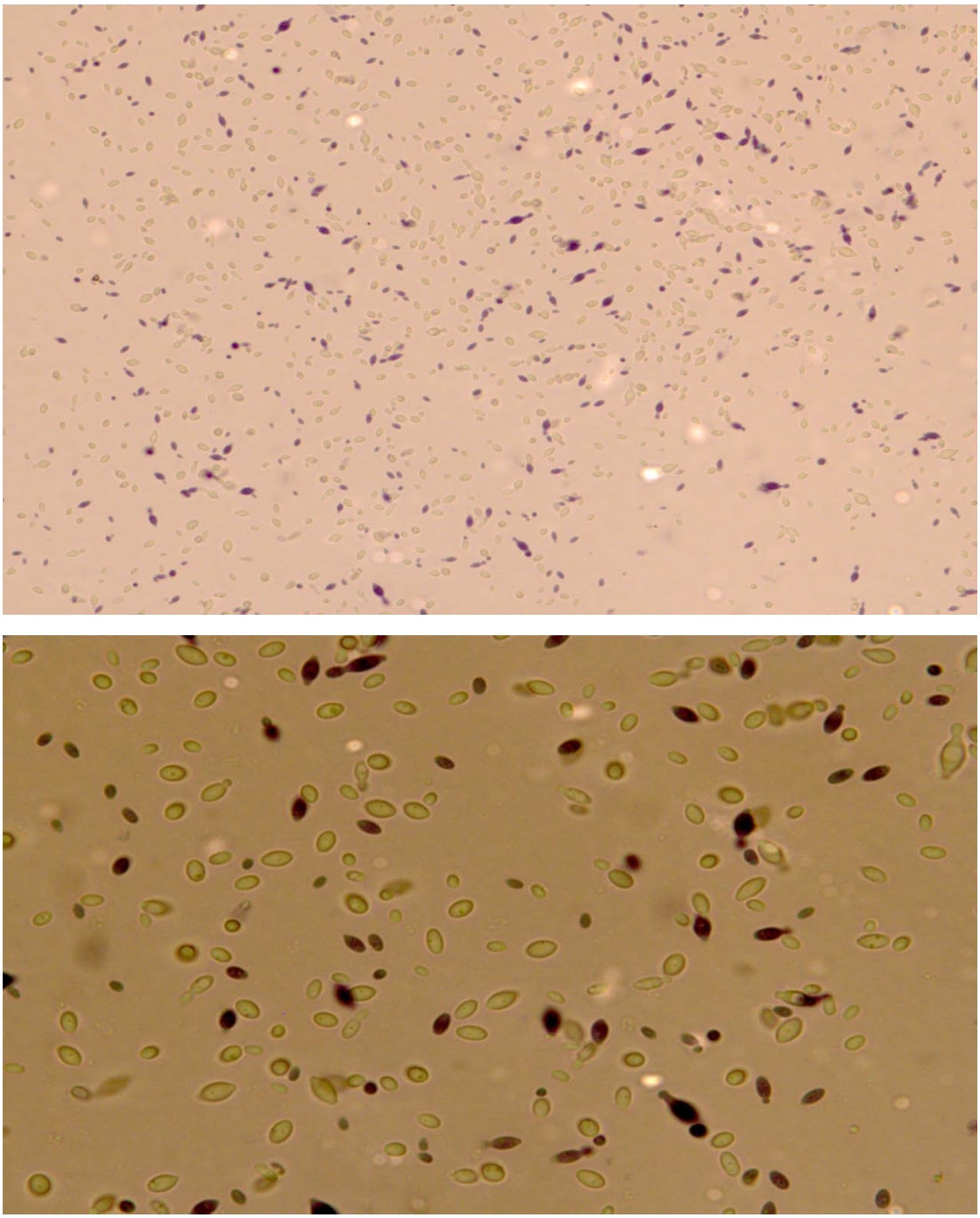


Figure A.1. Microscobic appearance of the microorganisms in fresh squeezed white grape juice.

APPENDIX B

PROPERTIES OF THE WHITE GRAPE JUICE SAMPLES

Table B.1. Some quality parameters after different amounts of L- ascorbic adding to the fresh squeed white grape Juice (before freezing).

Ascorbic acid (mg/l)	**pH**	**Turbidity (NTU)**	**Absorbance coefficient (cm^{-1})**	**Color**			
				L*	**a***	**b***	**(ΔE)**
normal	4.3	2052	33.33	40.7	-1.40	4.01	40.92
200	4.23	1130	26.61	32.45	-1.61	2.25	32.56
400	4.24	772	36.01	32.15	-1.51	1.93	32.24
600	4.25	878	48.03	34.20	-2.02	2.71	34.36

Table B.2. Density and viscosity data versus temperature of pasteurized and freshly squeezed white grape juice.

Temperature (°C)	Pasteurized white grape juice		Fresh squeezed white grape juice	
	Density (g/cm3)	Viscosity (cp)	Density (g/cm3)	Viscosity (cp)
5	1.066	3.029	1.0641	3.23
10	1.0709	2.589	1.0659	2.74
20	1.0731	1.986	1.0653	2.1
30	1.069	1.557	1.0608	1.68
40	1.0634	1.495	1.0558	1.43
50	1.0571	1.441	1.0510	1.40
60	1.05	1.370	1.0449	1.39
70	1.0441	1.277	1.0480	1.27
80	1.038	1.216		1.21
90		1.152		1.11

APPENDIX C

DATA OF THE CONTINUOUS FLOW UV STUDY

Table C.1. Logarithmic reduction data of *S. cerevisiae* in the pasteurized white grape juice at low flow rate (820 ml/min).

CYCLES	**LOGARITHMIC REDUCTION RATE (log N/No)**	**STANDART DEVIATION (log cfu/ml)**	**UV DOSE (mJ/cm^2)**
C1 IN	0	0	
C1 OUT	-0.14081	0.025105	7.007
C2 IN	-0.28129	0.141499	
C2 OUT	-0.47461	0.03969	14.014
C3 IN	-0.45542	0.024939	
C3 OUT	-1.03305	0.013973	21.021
C4 IN	-0.94258	0.006207	
C4 OUT	-1.72411	0.063796	28.028
C5 IN	-1.45085	0.024995	
C5 OUT	-1.87433	0.2724	35.035
C6 IN	-1.989	0.015552	
C6 OUT	-2.72666	0.02822	42.042
C7 IN	-2.48917	0.039742	
C7 OUT	-3.05882	0.030396	49.049
C8 IN	-2.67079	0.06946	
C8 OUT	3.392655	0.04446	56.056

Table C.2. Logarithmic reduction data of *S. cerevisiae* in the pasteurized white grape juice at medium flow rate (1520 ml/min).

CYCLES	**LOGARITHMIC REDUCTION RATE (log N/No)**	**STANDART DEVIATION (log cfu/ml)**	**UV DOSE (mJ/cm^2)**
C1 IN	0	0	
C1 OUT	-0.029	0.041006	2.884
C2 IN	-0.07133	0.03617	
C2 OUT	-0.11532	0.08979	5.768
C3 IN	-0.14715	0.08057	
C3 OUT	-0.30445	0.077177	8.652
C4 IN	-0.43483	0.052175	
C4 OUT	-0.71418	0.005963	11.536
C5 IN	-1.00342	0.077177	
C5 OUT	-0.98132	0.066059	14.42
C6 IN	-1.17552	0.091665	
C6 OUT	-1.3635	0.088345	17.304
C7 IN	-1.5	0.144335	
C7 OUT	-1.56085	0.102856	20.188
C8 IN	-1.66023	0.094426	
C8 OUT	-1.87373	0.00574	23.072

Table C.3. Logarithmic reduction data of *S. cerevisiae* in the pasteurized white grape juice at high flow rate (2280 ml/min).

CYCLES	**LOGARITHMIC REDUCTION RATE (log N/No)**	**STANDART DEVIATION (log cfu/ml)**	**UV DOSE (mJ/cm^2)**
C1 IN	0	0	
C1 OUT	-0.11829	0.022505	1.708
C2 IN	-0.26842	0.033952	
C2 OUT	-0.31266	0.008624	3.416
C3 IN	-0.49481	0.060514	
C3 OUT	-0.49552	0.078877	5.124
C4 IN	-0.71025	0.015956	
C4 OUT	-0.76556	0.066347	6.832
C5 IN	-1.16354	0.062319	
C5 OUT	-1.26458	0.043826	8.54
C6 IN	-1.60819	0.016399	
C6 OUT	-1.35818	0.029607	10.248
C7 IN	-1.75626	0.040864	
C7 OUT	-1.8167	0.022603	11.956
C8 IN	-1.99727	0.003863	
C8 OUT	-1.86963	0.072389	13.664

Table C.4. Logarithmic reduction data of spoilage microorganisms in the fresh squeezed white grape juice at low flow rate (774 ml/min).

CYCLES	**LOGARITHMIC REDUCTIONS (log cfu/ml)**				**UV DOSE (mJ/cm^2)**
	YEASTS	**STANDART DEVIATIONS**	**LACTIC ACID BACTERIA**	**STANDART DEVIATIONS**	
C1 IN	0	0	0	0	
C1 OUT	-0.09904	0.025561	-0.179302	0.020532	2.412
C2 IN	-0.11863	0.012292	0.028902	0.081496	
C2 OUT	-0.14364	0.011158	-0.147891	0.080503	4.824
C3 IN	-0.30148	0.010628	-0.351202	0.066098	
C3 OUT	-0.37007	0.025611	-0.419493	0.020731	7.236
C4 IN	-0.47031	0.031405	-0.438185	0.009163	
C4 OUT	-0.69153	0.010516	-0.473446	0.04582	9.648
C5 IN	-0.7219	0.00386	-0.686921	0.087234	
C5 OUT	-0.79974	0.01926	-0.942256	0.01646	12.06
C6 IN	-0.87054	0.098562	-1.071448	0.061081	
C6 OUT	-1.18046	0.024156	-1.533587	0.045034	14.472
C7 IN	-1.22969	0.013598	-1.419493	0.020731	
C7 OUT	-1.25719	0.002453	-1.547244	0.108766	16.884
C8 IN	-1.28633	0.025269	-1.582642	0.032394	
C8 OUT	-1.54267	0.034661	-1.640243	0.002577	19.296

Table C.5. Logarithmic reduction data of spoilage microorganisms in the fresh squeezed white grape juice at medium flow rate (1512 ml/min).

CYCLES	**LOGARITHMIC REDUCTIONS (log cfu/ml)**				**UV DOSE (mJ/cm^2)**
	YEASTS	**STANDART DEVIATIONS**	**LACTIC ACID BACTERIA**	**STANDART DEVIATIONS**	
C1 IN	0	0	0	0	
C1 OUT	-0.083166	0.022937	-0.13992	0.0211872	2.4948
C2 IN	-0.140579	0.04585	-0.18538	0.01696322	
C2 OUT	-0.172144	0.0630246	-0.267233	0.04247831	4.9896
C3 IN	-0.201577	0.0415999	-0.345741	0.04768108	
C3 OUT	-0.34192	0.0550296	-0.558262	0.00583423	7.4844
C4 IN	-0.403587	0.0677917	-0.85458	0.00282596	
C4 OUT	-0.434305	0.0337268	-0.993482	0.01178214	9.9792
C5 IN	-0.494638	0.0547387	-1.078719	0.00993612	
C5 OUT	-0.739338	0.0570883	-1.424033	0.00959973	12.474
C6 IN	-0.824458	0.0369154	-1.544895	0.01306974	
C6 OUT	-1.063406	0.0654685	-1.789986	0.07527529	14.9688
C7 IN	-1.152327	0.0424445	-1.868946	0.03039093	
C7 OUT	-1.416106	0.0563218	-2.11122	0.01056442	17.4636
C8 IN	-1.597111	0.0203029	-2.279306	0.01058315	
C8 OUT	-1.699235	0.0276757	-2.490306	0.00309676	19.9584

Table C.6. Logarithmic reduction data of spoilage microorganisms in the fresh squeezed white grape juice at high flow rate (2085 ml/min).

CYCLES	**LOGARITHMIC REDUCTIONS (log cfu/ml)**				**UV DOSE (mJ/cm^2)**
	YEASTS	**STANDART DEVIATIONS**	**LACTIC ACID BACTERIA**	**STANDART DEVIATIONS**	
C1 IN	0	0	0	0	
C1 OUT	-0.26281	0.104307	-0.16766	0.0972	1.001
C2 IN	-0.25985	0.071544	-0.1673	0.113595	
C2 OUT	-0.26105	0.002001	-0.11117	0.123316	2.002
C3 IN	0.066984	0.052355	-0.25421	0.105342	
C3 OUT	0.021395	0.053543	-0.11495	0.099111	3.003
C4 IN	0.095225	0.019686	-0.25287	0.091284	
C4 OUT	-0.05665	0.03695	-0.36716	0.115864	4.004
C5 IN	-0.08393	0.000548	-0.56841	0.086724	
C5 OUT	-0.07563	0.012285	-0.61717	0.086079	5.005
C6 IN	-0.1192	0.053076	-0.71509	0.107651	
C6 OUT	-0.23674	0.008902	-0.65117	0.082953	6.006
C7 IN	-0.31811	0.123967	-0.87641	0.096579	
C7 OUT	-0.36863	0.014623	-0.95184	0.045768	7.007
C8 IN	-0.56677	0.049908	-1.03169	0.109532	
C8 OUT	-0.4708	0.027268	-1.08406	0.11888	8.008

Table C.7. Two sample t-test on the pasteurized and fresh squeezed white grape juice.

Properties	**Pasteurized**			**Fresh squeezed**		
	low	**medium**	**high**	**low**	**medium**	**high**
pH	0.293	0.106	0.293	0.293	0.764	0.155
brix	0.000	0.000	0.001	0.049	0.698	0.002
density	0.000	0.003	0.001	0.020	0.423	0.006
turbidity	0.000	0.002	0.024	0.333	0.003	0.020
abs	0.000	0.000	0.000	0.000	0.000	0.000
T.A.	0.072	0.004	0.889	0.001	0.004	0.002
L*	0.017	0.120	0.026	0.011	0.019	0.279
a*	0.002	0.012	0.111	0.010	0.000	0.711
b*	0.628	0.039	0.001	0.017	0.000	0.012
BI	0.073	0.418	0.380	0.003	0.000	0.009

Significant: p value < 0.05