DEVELOPMENT OF DIFFERENT KORUK (UNRIPE GRAPE) PRODUCTS BY USING SEVERAL PROCESSING TECHNIQUES

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

DOCTOR OF PHILIOSOPHY

in Food Engineering

by
Zehra KAYA

July 2018
İZMİR

We approve the thesis of **Zehra KAYA**

Examining Committee Members:

Prof. Dr. Sevcan ÜNLÜTÜRK
Department of Food Engineering, İzmir Institute of Technology

---

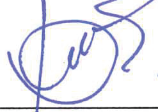
Prof. Dr. Figen KOREL
Department of Food Engineering, İzmir Institute of Technology

---

Assoc. Dr. Melike SAKİN YILMAZER
Department of Gastronomy and Culinary Arts, İzmir Katip Çelebi University

---

Prof. Dr. Banu ÖZEN
Department of Food Engineering, İzmir Institute of Technology



Prof. Dr. Duygu KIŞLA
Department of Food Engineering, Ege University

6 July 2018

Prof. Dr. Sevcan ÜNLÜTÜRK
Supervisor,
Department of Food Engineering,
İzmir Institute of Technology

Prof. Dr. Ahmet YEMENİCİOĞLU
Head of the Department of Food Engineering

Prof. Dr. Aysun SOFUOĞLU
Dean of the Graduate School of Engineering and Sciences

ACKNOWLEDGMENTS

Firstly, I would like to express my sincere and heartfelt appreciations to my supervisor Prof. Dr. Sevcan ÜNLÜTÜRK for her guidance, encouragement and endless support. I am very grateful to her for giving me opportunity to study with her throughout my Ph.D. research, it is an honour to work with her.

I would like express my special gratitude to my committee members, Prof. Dr. Figen KOREL and Assoc. Dr. Melike SAKİN YILMAZER, for their encouragements, valuable comments and recommendations during the progress meetings of my thesis for every 6 months. I would like to add my thankfullness Prof. Dr. Banu ÖZEN and Prof. Dr. Duygu KIŞLA for their special kindness in my thesis committee and precious suggestions in my thesis defence.

I would like to acknowledge the financial support from the Scientific and Technological Research Council of Turkey (TUBITAK) 2214-A International Doctoral Research Fellowship Programme for Chapter 5 of this thesis, which is related to PUV technology. For this chapter, I would like to also express my special thanks to Prof. Dr. Olga Martin-Belloso, Assoc. Dr. Robert Soliva-Fortuny and my lab-mates in Department of Food Technology in Lleida University, Spain.

Additinally, I'm deeply grateful to my unique lab-mates Dr. Semanur YILDIZ and Merve AKGÜN, and my special colleagues Kevser SABANCI and Ezgi EVCAN for the unforgettable and amusing moments even in my worst times. They are more than friends for me in the Office "221" during my IZTECH life. I also thank to Dr. Bengi HAKGÜDER TAZE, all specialists in BIOMER, Berk UYSAL, and Oğulcan ÖZDEMİR for their kind helps and supports. I would like to express my sincere appreciations to all the faculty members and friends for everything I have learned in this area.

Finally, 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, also my colleague, İbrahim KAYA, who promoted me to be a food engineer. I always listen and admire him during my life. I would never been able to achieve without my dear mother Ferah KAYA and her endless patience and advice, thanks her for everything. And of course, I am very lucky to have a wonderful sister, as well as a Ph.D candidate of civil engineering, my precious, Derya KAYA. I heartily thank her for being the most important supporter of my academic and social life and for being my best friend in my life.

ABSTRACT

DEVELOPMENT OF DIFFERENT KORUK (UNRIPE GRAPE) PRODUCTS BY USING SEVERAL PROCESSING TECHNIQUES

Verjuice, obtained by pressing unripe grapes, is mostly used as an alternative to lemon juice and vinegar in salads and traditional meals due to its unique sour taste and flavour. It has a short shelf life due to growth of yeasts and molds when produced at household conditions. Verjuice products in the market are not meeting desirable quality characteristics. The high quality verjuice and its derivatives required to be produced by considering several processing techniques.

The objectives of this Ph.D. thesis are to (i) produce long shelf life verjuice with minimum change in its "fresh like" properties by using the combination of UV-C irradiation or Pulsed-UV light (PUV) with mild heating, (ii) produce verjuice powder with a good storage stability by using freeze drying, (iii) produce concentrated verjuice with minimal quality loss by using vacuum evaporation.

Verjuice was successfully pasteurized without loosing its desirable quality by a combined UV-C and mild heating (78.0 mJ/mL, 6.2 min, 51.2°C) and PUV assisted with mild heating (6.12 J/cm^2, 8.5 min, 47°C) and mild thermal (72°C, 18 s) treatments. 5-log reduction of target *S. cerevisiae* (NRRL Y-139) was aimed for the pasteurization of verjuice. No microorganism was developed in pasteurized verjuice during 12 and 6 weeks of refrigerated storage after UV+MH and PUV+MH treatments, respectively. Freeze-dried verjuice powder containing maltodextrin (FD 48h, 20% MD), was highly stable under the accelerated storage conditions (40°C, 90%RH) for 70 days. Concentrated verjuice obtained under vacuum conditions of 45°C/913 mbar, showed minimal quality change and was consumable up to 12 weeks at refrigerated storage.

ÖZET

ÇEŞİTLİ İŞLEME TEKNİKLERİ KULLANARAK FARKLI KORUK (OLGUNLAŞMAMIŞ ÜZÜM) ÜRÜNLERİNİN GELİŞTİRİLMESİ

Olgunlaşmamış üzümlerin sıkılmasıyla elde edilen koruk suyu, kendine has ekşi tat ve lezzete sahip olmasından dolayı genellikle salata ve geleneksel yemeklerde, limon suyu ve sirkeye alternatif olarak kullanılmaktadır. Koruk suyu, ev ortamında üretildiğinde maya ve küflerin gelişmesi nedeniyle kısa bir raf ömrüne sahiptir. Piyasadaki koruk suyu kullanılarak elde edilen ürünler istenen kalite özelliklerini karşılamamaktadır. Çeşitli işleme teknikleri dikkate alınarak yüksek kalitede koruk suyu ve ürünleri üretilmesi gerekmektedir.

Bu doktora tezinin amacı, (i) UV-C ışınlama veya darbeli ışık (PUV) gibi ısısal olmayan teknolojilerin hafif ısısal işlem ile kombinasyonu kullanılarak "taze benzeri" özelliğinde minimum değişimle uzun raf ömrüne sahip koruk suyu; (ii) dondurarak kurutma tekniği kullanılarak depolama dayanıklılığı iyi olan koruk suyu tozu; (iii) vakumlu buharlaştırmayla kalite kaybı en az olan koruk suyu konsantresi üretmektedir.

Taze sıkılmış koruk suyu, UV-C ve hafif ısısal işlemin birleştirilmesi (78.0 mJ/mL, 6.2 dk, 51.2°C), atımlı ışığın hafif ısıda uygulanması (6.12 J/cm^2, 8.5 dk, 47°C) ve ısısal pastörizasyon (72 °C, 18 s) ile istenen kalite özelliklerini kaybetmeden başarıyla pastörize edilmiştir. Koruk suyu pastörizasyonu için *S. cerevisiae* (NRRL Y-139) hücrelerinin 5-log azalması hedeflenmiştir. Pastörize edilen koruk suyu, UV+MH ve PUV+MH uygulamalarından sırasıyla 12 ve 6 hafta soğuk depolama sonrasında, minimum kalite kaybı ile mikrobiyolojik olarak güvenli olmuştur. 45°C/914 mbar vakum koşullarında üretilen konsantre verjuice, soğutulmuş depoda 12 haftaya kadar, kalite özelliklerinin ve HMF miktarının minimum değişimi ile tüketilebilir durumdadır. Bir taşıyıcı olarak maltodekstrin ilave edilerek dondurularak elde edilen kurutulmuş koruk suyu tozu (FD 48h, 20% MD), 40°C ve %90 bağıl nemdeki hızlandırılmış depolama koşullarında bile 70 gün boyunca oldukça kararlıdır.

This Ph.D. thesis is dedicated to my dear parents,

Ibrahim KAYA and Ferah KAYA,

and my precious sister,

Derya KAYA,

who are the biggest supporters in my life.

I love you too much!

TABLE OF CONTENTS

LIST OF FIGURES……………………………………………………………………xiv

LIST OF TABLES…………………………………………………………..…..xviii

LIST OF SYMBOLS AND ABBREVIATIONS……………………………………xxi

CHAPTER 1. INTRODUCTION 1

CHAPTER 2. LITERATURE REVIEW 6

2.1. Introduction 6

2.2. Fruit Juice 7

2.2.1. Nutritive Value and Composition of Fruits and Fruit Juices 7

2.2.2. Microbial Spoilage of Fruit Juice and Regulatory Issues 9

2.3. Preservation Methods of Fruit Juices 12

2.3.1. Thermal Pasteurization 13

2.3.2. Nonthermal Preservation Techniques 14

2.3.2.1. Ultraviolet Light (UV-C) Irradiation 17

2.3.2.2. Pulsed-UV (PUV) Light Technology 21

2.3.2.3. Hurdle technology 22

2.4. Fruit Juice Powder 25

2.4.1. Drying Methods Used for Fruit Juice Powder 26

2.4.1.1. Freeze Drying 28

2.4.2. The Effect of Different Drying Methods on the Quality Properties of Fruit Juice Powder 31

2.5. Concentrated Fruit Juice 34

2.5.1. Evaporation Methods Used Fruit Juice Concentrates 34

2.5.2. Effect of Evaporation Methods on the Quality Properties of Concentrated Fruit Juice 38

2.6. Grape and Verjuice 41

2.6.1. The Production of Grape in the World and Turkey 41
2.6.2. Raw Material of Verjuice: Unripe Grape (Koruk) 45
2.6.3. Verjuice 45
2.6.4. Verjuice Powder 47
2.6.5. Concentrated Verjuice 47
2.6.5.1. The Potential of Verjuice Products in the Market 48

CHAPTER 3. VERJUICE PASTEURIZATION BY ULTRAVIOLET IRRADIATION (UV-C) TREATMENT 50
3.1. Materials and Methods 51
3.1.1. Raw material: Koruk (Unripe Grape) 51
3.1.2. Verjuice Preparation 51
3.1.3. Verjuice Characterization 52
3.1.4. Microbiological Analysis 53
3.1.4.1. Background Microflora of Verjuice 53
3.1.4.2. Target Microorganism, Sample Inoculation and Enumeration 53
3.1.5. Experimental Procedure 56
3.1.6. UV-C Irradiation of Verjuice (UV) 56
3.1.7. Mild Heating (MH) 60
3.1.8. UV-C Irradiation Assisted with Mild Heating (UV+MH) 60
3.1.9. UV dose calculation: Actinometrical Study 61
3.1.10. Modelling of UV-C Inactivation Kinetics of *S. cerevisiae* in verjuice 63
3.1.10.1. Calculation of Synergistic Effect of UV-C Irradiation and Mild Heating 67
3.1.11. Data Analysis 67
3.2. Results and Discussion 69
3.2.1. Characterization of the Freshly Squeezed Verjuice 69
3.2.2. The Effect of UV-C Irradiation (UV) on *S. cerevisiae* in Verjuice 70

3.2.3. The Effect of Mild Heating (MH) on *S. cerevisiae* in Verjuice ... 72
3.2.4. The Effect of Combined Treatment (UV+MH) on *S. cerevisiae* in Verjuice ... 74
3.2.5. Modelling and Kinetics of *S. cerevisiae* Inactivation in verjuice by UV, MH and UV+MH processes ... 76
3.2.6. Synergistic lethal effect of Combined UV+MH Treatment ... 80
3.3. Conclusions ... 81

CHAPTER 4. SHELF LIFE OF VERJUICE PASTEURIZED BY A COMBINED UVC-MILD HEAT TREATMENT ... 83
4.1. Materials and Methods ... 84
4.1.1. Conventional Thermal Pasteurization (P) ... 84
4.1.1.1. Determination of Thermal Inactivation Kinetics Parameters of *S. cerevisiae* in Verjuice ... 84
4.1.1.2. Thermal Pasteurization of Verjuice ... 87
4.1.2. Pasteurization with Combined UV-C -Mild Heat Treatment (UV+MH2) ... 88
4.1.3. Storage of Verjuice ... 89
4.1.4. Microbiological Analyses ... 89
4.1.5. Physicochemical Analyses and Optical Properties of Verjuice ... 90
4.1.5.1. pH ... 90
4.1.5.2. Total Soluble Solid Content ... 90
4.1.5.3. Titratable acidity ... 92
4.1.5.4. Absorption coefficient ... 92
4.1.5.5. Turbidity ... 92
4.1.5.6. Color ... 93
4.1.6. Data Analysis ... 94
4.2. Results and Discussion ... 94
4.2.1. Effect of Combined UVC-Mild Heat (UV+MH2) Treatment on the Quality Properties of Verjuice ... 94

4.2.2. Effect of Combined UV+MH2 Treatment on the Quality Properties of Verjuice During Storage 98
4.2.2.1. Microbiological Properties of Verjuice 98
4.2.2.1. Physicochemical and Optical Properties of Verjuice during Storage 101
4.3. Conclusions 112

CHAPTER 5. VERJUICE PASTEURIZATION BY PULSED-UV LIGHT (PUV) TREATMENT 114
5.1. Materials and Methods 116
5.1.1. Raw Materials: Unripe Grapes and Verjuice Preparation 116
5.1.2. Verjuice Characterization 116
5.1.2.1. Antioxidant Activity of Verjuice 117
5.1.3. Microbiological Analysis: Background Microflora, Target Microorganism, Sample Inoculation and Enumeration 117
5.1.4. Experimental Procedure of Microbial Inactivation Study 118
5.1.4.1. Pulsed-UV Light (PUV) Treatment of Verjuice 121
5.1.4.2. Mild Heating (MH) 122
5.1.4.3. Pulsed-UV Light Assisted with Mild Heating (PUV+MH) 124
5.1.4.4. Dose Calculation of Pulsed-UV Light (PUV) 124
5.1.5. Shelf Life Study 125
5.1.5.1. Thermal Pasteurization of Verjuice 125
5.1.5.2. Combined Pulsed-UV Light and Mild Heating (PUV+MH) Pasteurization of Verjuice 126
5.1.5.3. Storage and Analyses of Verjuice during its Shelf Life 127
5.1.6. Data Analyses 127
5.2. Results and Discussion 129
5.2.1. Characterization of Verjuice 129

5.2.2. The Effect of Pulsed-UV Light (PUV) on *S. cerevisiae* Inoculated into Verjuice 130
5.2.3. The Effect of Mild Heating (MH) on *S. cerevisiae* Inoculated into Verjuice 133
5.2.4. The Effect of Combined PUV-Mild Heat (PUV+MH) Treatment on *S. cerevisiae* Inoculated into Verjuice 135
5.2.5. The Shelf Life of Verjuice Pasteurized by Combined Pulsed-UV Light–Mild Heating (PUV+MH) 137
5.2.5.1. The Quality Properties of Verjuice Pasteurized by Combined Pulsed-UV Light–Mild Heating at 47 °C (PUV+MH47) 137
5.2.5.2. Effect of Combined PUV+MH47 Treatment on the Quality Properties of Verjuice During Storage 141
5.3. Conclusions 152

CHAPTER 6. PRODUCTION OF VERJUICE POWDER 155
6.1. Materials and Methods 156
6.1.1. Freeze Drying of Verjuice 156
6.1.2. Powder Analyses 158
6.1.2.1. Product Yield or Product Recovery 158
6.1.2.2. Moisture Content 159
6.1.2.3. Water Activity 159
6.1.2.4. Bulk and Tapped Density 159
6.1.2.5. Solubility 160
6.1.2.6. Hygroscopicity 160
6.1.2.7. Color Analyses 161
6.1.2.8. Thermal properties 161
6.1.2.9. Morphology/Microstructure of Particles 162
6.1.3. Accelerated Shelf Life Study 162
6.1.4. Statistical Analysis 164
6.2. Results and Discussion 165
6.2.1. Product Yield of the Freeze Drying Process 165

6.2.2. Moisture Content and Water Activity of Verjuice Powders ... 166
6.2.3. Physical Properties of Verjuice Powder ... 168
6.2.4. Color Analyses of Verjuice Powders ... 173
6.2.5. Thermal Properties of Verjuice Powders ... 177
6.2.6. Microstructure of Verjuice Powders ... 178
6.2.7. Changes in The Quality of Verjuice Powders during Accelerated Storage ... 181
6.3. Conclusions ... 188

CHAPTER 7. PRODUCTION OF CONCENTRATED VERJUICE ... 190
7.1. Materials and Methods ... 191
7.1.1. Concentration Process of Verjuice ... 191
7.1.2. Evaporation Rate ... 193
7.1.3. Physicochemical Analyses of the Concentrated Verjuice ... 194
7.1.3.1. Density ... 194
7.1.3.2. Viscosity ... 194
7.1.3.3. Total Phenolic Content ... 195
7.1.3.4. Non enzymatic browning index (NEBI) ... 196
7.1.3.5. Hydroxymethylfurfural (HMF) ... 196
7.1.4. Shelf Life Study ... 197
7.1.4.1. Kinetic modelling of color parameters during storage ... 197
7.1.5. Statistical Analysis ... 198
7.2. Results and Discussion ... 198
7.2.1. Evaporation Rate of Verjuice ... 198
7.2.2. Physicochemical Properties of Concentrated Verjuice ... 201
7.2.3. Color Analyses of Concentrated Verjuice ... 203
7.2.4. Total Phenolic Content of Concentrated Verjuice ... 206
7.2.5. NEBI and HMF of Concentrated Verjuice ... 208
7.2.6. Changes in the Quallity of Concentrated Verjuice during Shelf Life ... 210

7.2.6.1. Kinetic Modelling in Color of the Concentrated Verjuice during Shelf Life 217
7.3. Conclusions 219

CHAPTER 8. CONCLUSION 221

REFERENCES 225

APPENDICES
APPENDIX A. VERJUICE PRODUCTION USING ULTRAVIOLET (UV-C) PASTEURIZATION 257
APPENDIX B. VERJUICE PASTEURIZATION BY PULSED-UV LIGHT (PUV) TREATMENT 263
APPENDIX C. PRODUCTION OF VERJUICE POWDER 264
APPENDIX D. PRODUCTION OF CONCENTRATED VERJUICE 266

LIST OF FIGURES

Figure **Page**

Figure 2.1. The electromagnetic spectrum 17
Figure 2.2. DNA structure after UV-C irradiation 18
Figure 2.3. Types of hurdle effects 24
Figure 2.4. Basic configuration of freeze dryer 29
Figure 2.5. Basic configuration of vacuum evaporation equipment 38
Figure 2.6. Grape production in the world in 2016 41
Figure 2.7. Grape exporting countries in the world in 2016 42
Figure 2.8. Production of grape in Turkey in 2017 44
Figure 2.9. Use of grape in Turkey in 2017 44
Figure 2.10. Sour grape juice (a) and sour grape sauce (b) 49
Figure 3.1. Flow diagram of verjuice production 52
Figure 3.2. Viability of *S. cerevisiae* (NRRL Y-139) in freshly squeezed verjuice before and after acid adaptation 55
Figure 3.3. The flow diagram of inactivation studies conducted in Chapter 3 57
Figure 3.4. Laboratory Scale Continuous Flow UV-C Reactor System 58
Figure 3.5. Dimensions of the UV-C reactor (a & b) and arrangements of the 7 UV-C lamps in the processes (c: Black circle: open lamp, empty circle: closed lamp) 59
Figure 3.6. Several types of microbial inactivation curves 64
Figure 3.7. Typical survival curve of UV exposed microorganisms 65
Figure 3.8. Inactivation of acid adapted *S. cerevisi*ae (NRRL Y-139) at low (▲) and high (■) initial load in freshly squeezed verjuice by using UV-C irradiation 72
Figure 3.9. Inactivation of acid adapted *S. cerevisiae* (NRRL Y-139) cells in freshly squeezed verjuice by using mild heating 73
Figure 3.10. Inactivation of acid adapted *S. cerevisiae* (NRRL Y-139) cells in freshly squeezed verjuice by using UV-C irradiation combined with mild heating 75

Figure 3.11. The required pasteurization time (5D) for inactivation of *S.cerevisiae* (NRRL Y-139) in verjuice subjected to UV-C (UV), mild heating (MH1 & MH2) and combined processes (UV+MH1 & UV+MH2) 81
Figure 4.1. Thermal Death Time (TDT) curves for calculation of D (a) and z (b) values 85
Figure 4.2. Continuous Heat Pasteurization System 88
Figure 4.3. Diagram of the shelf life study of freshly squeezed verjuice 91
Figure 4.4. CIELAB color space 93
Figure 4.5. Total color difference (ΔE) and browning index (BI) of untreated (U), treated with combined UV-C and mild heating (UV+MH2) and thermally pasteurized (P) verjuice 97
Figure 4.6. Changes in yeasts and molds (YM), and total mesophilic aerobic counts (TMAC) in (freshly squeezed verjuice (U) during storage 99
Figure 4.7. Changes in physicochemical properties of untreated (U: circle), combined treated (UV+MH2: triangle) and thermally pasteurized (P: square) verjuice during 12 weeks at refrigerated storage 104
Figure 4.8. Changes in color of untreated (U: circle), treated with combination of processes (UV+MH2: triangle) and thermally (P: square) pasteurized verjuice during 12 weeks at refrigerated storage 110
Figure 5.1. The viability of *S. cerevisiae* (NRRL Y-139) in verjuice before and after acid adaptation 119
Figure 5.2. The flow diagram of microbial inactivation studies conducted in Chapter 5 120
Figure 5.3. Schematic view of Laboratory Scale Bench Top Pulse Light System 121
Figure 5.4. Continuous Heat Pasteurization System used in Chapter 5 126
Figure 5.5. Diagram of the shelf life study of freshly squeezed verjuice 128
Figure 5.6. The inactivation of acid-adapted *S.cerevisiae* (NRRL Y-139) in verjuice by PUV treatment at different lamp distance 5 cm (a) and 10 cm (b) (juice depth Δ: 1mm, □:3mm, O:5mm) 132
Figure 5.7. The inactivation of acid-adapted *S.cerevisiae* Y139 in verjuice by MH treatment 134
Figure 5.8. The inactivation of acid-adapted *S.cerevisiae* in verjuice by PUV+MH treatment at different juice depth 136

Figure 5.9. Antioxidant activity (AA) (a), Total color difference (ΔE) (b) and browning index (BI) (c) of untreated (U), treated with combined PUV-mild heat (PUV+MH47) and thermally pasteurized (P) verjuice 140
Figure 5.10. Change in physicochemical properties of untreated (U: circle), treated with combination of processes (PUV+MH47: triangle) and thermally pasteurized (P: square) verjuice during 6 weeks at storage of 4°C and 25 °C 146
Figure 5.11. Changes in color of untreated (U: circle), combined treated (PUV+MH47: triangle) and thermally (P: square) pasteurized verjuice during 6 weeks at storage of 4 °C and 25 °C 149
Figure 6.1. The schematic diagram of production and storage of verjuice powder 157
Figure 6.2. Product yield (%) of freeze drying for verjuice powders 166
Figure 6.3. Solubility and hygroscopicity of verjuice powders after freeze drying 172
Figure 6.4. The change in color parameters of verjuice powder after freeze drying 176
Figure 6.5. Glass transition temperature (T_g) of verjuice powders after freeze drying 178
Figure 6.6. Scanning Electron Micrographs of verjuice powder after freeze drying for 48h and 72h (magnification at 100x) 179
Figure 6.7. Scanning Electron Micrographs of verjuice powder after freeze drying for 48h and 72h (magnification at 1000x) 180
Figure 6.8. Changes in the physical properties of freeze-dried verjuice powder* during the accelerated storage (40 °C/90% RH) of 70 days 183
Figure 6.9. Changes in the color properties of freeze-dried verjuice powder* during the accelerated storage (40 °C/90% RH) of 70 days (*: Powders obtained by drying J+20% MD feed mixture for 48 hours, FD 48h) 186
Figure 7.1. The schematic diagram of production of the concentrated verjuice 192
Figure 7.2. Change in verjuice concentrate (°Brix) produced by various concentration processes 199

Figure 7.3. Change in evaporation rate (grey bar) and evaporation rate constant (black line) of verjuice concentrate produced by various concentration processes 201
Figure 7.4. The change in color parameters of concentrated verjuice* produced by different evaporation processes.. 204
Figure 7.5. The change in the amount of TPC of concentrated verjuice* obtained by different evaporation processes... 207
Figure 7.6. Changes in NEBI and the amount of HMF in concentrated verjuice obtained by different evaporation processes (a: NEBI, b:HMF) 209
Figure 7.7. Changes in TPC of concentrated verjuice stored under refrigerated (4 °C) and room (25 °C) conditions for 12 weeks 212
Figure 7.8. Change in color parameters of concentrated verjuice stored in refrigerated (4 °C) and room (25 °C) conditions for 12 weeks................. 213
Figure 7.9. Changes in NEBI and the amount of HMF in concentrated verjuice stored in 4 °C and 25 °C for 12 weeks.. 216

LIST OF TABLES

Table **Page**

Table 2.1. Common juice definitions 7
Table 2.2. Composition of edible part of fruits (fresh wb) 8
Table 2.3. Juice Safety and Deterioration Hazards 10
Table 2.4. Growth conditions of foodborne pathogenic bacteria 11
Table 2.5. Thermal processing for fruit juice pasteurization 13
Table 2.6. The effect of thermal processing treatments on the fruit juice quality 15
Table 2.7. The advantages and disadvantages of minimally processed juice 16
Table 2.8. UV-C irradiation treatment of fruit juices 20
Table 2.9. Pulsed-UV light processing of fruit juices 23
Table 2.10. Drying methods used for production of fruit juice powder 27
Table 2.11. The summary of the freeze drying studies of fruit juice 33
Table 2.12. Evaporation methods used for production of concentrated fruit juice 35
Table 2.13. The summary of the vacuum evaporation studies of fruit juice 40
Table 2.14. The grape market in Turkey 43
Table 2.15. The main ingredients in verjuice products sold in the market 49
Table 3.1. Physicochemical and optical properties of freshly squeezed verjuice 70
Table 3.2. Goodness-of-fit (RMSE, Bf and Af) and model overfitting (AIC, BIC) parameters for the log-linear, log linear plus shoulder, log linear plus tail and Weibull models 77
Table 3.3. Pasteurization time (5D values) for inactivation of *S. cerevisiae* (NRRL Y-139) in freshly squeezed verjuice calculated by means of Weibull Model and synergism of processes 79
Table 4.1. Different temperature and time combinations for D and z values calculations 87
Table 4.2. Physicochemical and optical properties of untreated (U), treated with combined UV-C and mild heating (UV+MH2) and thermally pasteurized (P) verjuice. 95

Table 4.3. Color properties of untreated (U), treated with combined UV-C and mild heating (UV+MH2) and thermally pasteurized (P) verjuice. 97
Table 4.4. Microbial quality of verjuice which was untreated (U), and treated with combined (UV+MH2) and thermal pasteurization (P) during storage 99
Table 4.5. Changes in physicochemical properties of verjuice during 12 weeks of refrigerated storage 102
Table 4.6. Changes in color properties of verjuice during 12 weeks at refrigerated storage 109
Table 5.1 PUV treatment conditions used in this chapter 122
Table 5.2. MH and PUV+MH treatment conditions used in the chapter 123
Table 5.3. Physicochemical and optical properties of verjuice 130
Table 5.4. The log reductions of acid-adapted *S.cerevisiae* (NRRL Y-139) inoculated into verjuice by PUV treatment at different lamp distances and juice depth 133
Table 5.5. The log reductions of acid-adapted *S.cerevisiae* (NRRL Y-139) in verjuice by PUV+MH treatment at different temperatures and juice depth 135
Table 5.6. Physicochemical and optical properties of untreated (U), treated with combined PUV-mild heat (PUV+MH47) and thermally pasteurized (P) verjuice 138
Table 5.7. Color properties of untreated (U), treated with combined PUV-mild heat (PUV+MH47) and thermally pasteurized (P) verjuice 139
Table 5.8. The effect of storage (4 °C) on the physicochemical, optical and antioxidant properties of untreated verjuice & verjuice subjected to different treatments 144
Table 5.9. The effect of storage (25 °C) on the physicochemical, optical and antioxidant properties of untreated verjuice & verjuice subjected to different treatments 145
Table 6.1. Freeze drying experiment conditions 158
Table 6.2. Moisture content and water activity of verjuice powders after freeze drying 167
Table 6.3. Physical properties of verjuice powders after freeze drying 169

Table 6.4. Color properties of verjuice powders after freeze drying 175
Table 7.1. Processing conditions of verjuice evaporation 193
Table 7.2. Evaporation rate and evaporation rate constant of verjuice during evaporation processes at different conditions 200
Table 7.3. Physicochemical properties of concentrated verjuice produced by various evaporation processes... 202
Table 7.4. Color parameters of concentrated verjuice* produced by different evaporation processes 204
Table 7.5. The amount of TPC of concentrated verjuice produced by different evaporation processes.. 207
Table 7.6. Changes in physical properties of concentrated verjuice after 12 weeks of storage at refrigerated (4 °C) and room (25 °C) conditions 211
Table 7.7. Kinetic parameters of zero order (a) and first-order (b) equations for changes in the color of concentrated verjuice during refrigerated and room conditions storage 218

LIST OF SYMBOLS AND ABBREVIATIONS

ΔE	Total color difference
a*	Redness-grenness
A_e	Absorption coefficient (cm^{-1})
Af	Accuracy factor
a_w	Water activity
b*	Yellowness-blueness
Bf	Bias factor
BI	Browning index
C*	Chroma
cal	Calculation data
CFU	Colony forming unit
CI	Carr Index
c_{iodide}	Concentration of iodide
$c_{triiodide}$	Concentration of triiodide
D	Decimal reduction time (s, min)
E	Number of einsteins of photons absorbed by sample (Einstein/L)
exp	Experimental data
FD	Freeze Drying
GAE	Gallic acid equivalent
h°	Hue angle
HG	Hygroscopicity
HMF	Hydroxymethylfurfural
HR	Hausner Ratio
I_0	Incident UV intensity
I_{avg}	Average UV intensity absorbed by sample
J	Verjuice
k	Inactivation rate constant (1/s)
k_{max}	Inactivation rate constant of exponential death region
l	Path lenght (width) of the quartz quvette (cm)
L*	Lightness-Darkness
M	Molarity

MD	Maltodextrin
MH	Mild heating
N	Number of survival count (CFU/mL)
N_0	Initial microbial count (CFU/mL)
NEBI	Non-enzymatic browning index
N_{res}	Residual number of subpopulations (CFU/mL)
$n_{triiodide}$	Moles of triiodide
NTU	Nephelometric turbidity unit
OD	Optical density (absorbance)
P	Thermal pasteurization
p	shape parameter
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
PUV	Pulsed-UV light
PUV+MH	Combination of pulsed-UV light and mild heating
R^2	Coefficient of determination
RH	Relative Humidity
RMSE	Root mean square error
SI	Shoulder length time before exponential inactivation
t	Time (s)
T	Temperature (°C)
T.A.	Titratable acidity
TAMC	Total aerobic mesophilic bacteria count
TB	Traditional Boiling
TC	Total coliforms
TDT	Thermal death time
T_g	Glass transition temperature
T_{juice}	Juice inlet temperature
TPC	Total phenolic content
t_R	Reliable time or reliable life
TSS	Total soluble solid (°Brix)
t_{total}	Total processing time
t_{uv}	UV exposure time (t_2)

$T_{water\ circulation}$	Water temperature during circulation
U	Untreated
U.S. FDA	The United States Food and Drug Administration
UV	UV-C irradiation
UV+MH	Combination of ultraviolet irradiation and mild heating
UV-C	Short wave ultraviolet
VE	Vacuum Evaporation
VRBA	Violet Red Bile Agar
V_{sample}	Sample volume in the annular region of UV reactor
WSI	Water solubility index
YMC	Yeasts and mould count
YPD	Yeast extract-Peptone-Dextrose
δ	scale parameter
ε	Molar extinction coefficient (1.061 $M^{-1}*cm^{-1}$)
Φ	Quantum yield
k_0	Zero-order reaction rate constant
k_1	First-order reaction rate constant

CHAPTER 1

INTRODUCTION

Verjuice (green juice) is an unripe grape juice, which is obtained by pressing green grapes (koruk) grown especially in Mediterranian and Southeastern Anatolia region. Due to grape variability, it has a specific odour and sour taste (Hayoglu, Kola, Kaya, Ozer, & Turkoglu, 2009). Verjuice is also a savoury alternative to vinegar and lemon juice because it has the same acid-base equilibrium (Oncul & Karabiyikli, 2015). It is used generally in traditional meals, vegetable salads and snacks to give flavour, and mixed as an ingredient in several alcoholic beverages and sauces (Hildebrandt & Matchuk, 2002). Verjuice has a short shelf life because it is generally produced without any technological application at household conditions. Thus, it can be easily spoiled by means of microorganisms grown naturally in grapes. Additionally, turbidity of verjuice increases after spoilage and quality loss occurs due to sedimentation of particles during storage (Hayoglu et al., 2009).

Thermal treatment is the most common preservation method applied between 60 and 100 °C for a few seconds for pasteurization of fruit juice, with a pH lower than 4.5 in order to destroy vegetative pathogens, spoilage microorganisms and prevent enzyme inactivation (Rivas, Rodrigo, Martinez, Barbosa-Canovas, & Rodrigo, 2006). Food and Drug Administration (FDA) defines the pasteurization as "it is the required temperature and time combination for 5 log reduction of the target microorganisms" (U.S. FDA, 2001). Although thermal pasteurization provides long and stable shelf life, it has many unfavourable effects on sensory and nutritive quality of the food such as dramatic loss of flavour and taste, degradation of nutrients and undesirable browning reactions (Garde-Cerdan, Arias-Gil, Marselles-Fontanet, Ancin-Azpilicueta, & Martin-Belloso, 2007; Walkling-Ribeiro et al., 2008). UV-C irradiation that is a "nonthermal pasteurization process" can be used as an alternative to thermal pasteurization which may provide microbial safety and prolong the shelf life without causing any change in the nutritional and sensorial quality of the juices (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; Butz & Tauscher, 2002). UV-C irradiation have been used efficiently to inactivate foodborne pathogens in liquid foods since 29 November 2000 with and approval of Food and Drug Administration (U.S. FDA, 2000). UV-C light between 200 and 280 nm

wavelength (especially 253.7 nm), has a germicidal effect on microorganisms and prevent transcription and reproduction of the cells by passing from their cell wall and forming dimerization on thymine structure in their DNA (Bintsis et al., 2000). Recent studies showed that UV-C irradiation is successively used for fruit juice pasteurization in addition to water and air disinfection, and pathogen inactivation in meat, poultry and fish products (Koutchma, Forney, & Moraru, 2009). Pulsed-UV (PUV) technology is another nonthermal processing method to inactivate pathogens and spoilage microorganisms in foods. Decontamination of foods is ensured by using short time (1μs to 0.1s) high-peak pulses of broad spectrum wavelengths between 200 nm-1100 nm (Dunn, Ott, & Clark, 1995; Oms-Oliu, Martin-Belloso, & Soliva-Fortuny, 2010b). Microbial inactivation achieved by PUV can be explained by different mechanisms such as photochemical (DNA damage by absorbing UV-C light), photothermal/photophysical effect (overheating of the cell by absorbing high energy and destruction of the cell membrane) on the cell. It is found that Ultraviolet-C (UV-C) region of the Pulse Light (200-280 nm) has a great impact on the inactivation of microorganisms (Gomez-Lopez, Ragaert, Debevere, & Devlieghere, 2007; Koutchma, 2008). Food and Drug Administration (FDA) has been approved the use of Pulse Light on the food and food surfaces (U.S. FDA, 1996). PUV could be a promising alternative method for the decontamination of several solid foods such as meats, vegetables and fruits as well as liquid foods such as fruit juices (Gomez-Lopez et al., 2007; Oms-Oliu et al., 2010b).

Drying process is one of the most effective, inexpensive and common preservation methods that is applied to extend the shelf life of foods (Mishra, Mishra, & Mahanta, 2014; Ratti, 2001). Foods such as fruit juices are dried in order to reduce their weight, volume, and the size of their packages, provide easier transport and storage stability as well as economic benefits (Goula & Adamopoulos, 2010). The aim of drying is not only to extend the shelf life but also to preserve the quality of the fruit juice (Mani, Jaya, & Das, 2002). Fruit juice powders can be used in the food and pharmacological industry as flavour, aroma and colouring agent (Fazaeli, Emam-Djomeh, Ashtari, & Omid, 2012; Goula & Adamopoulos, 2010; Shrestha, Ua-Arak, Adhikari, Howes, & Bhandari, 2007). Several drying methods are applied to foods including conventional air drying, spray drying and freeze drying. Among these methods, the freeze drying removes the water from the juice in the ice form by sublimation (Franks, 1998). The excellent nutritional and sensorial quality of the product is obtained by freeze drying due to low temperature operation (Erbay & Kucukoner, 2008; Ratti, 2001). However, powders obtained from

fruits include high amount of sugars having low molecular weight and low glass transition temperature (Tg). Thus, three types of powder behavior i.e., collapsing, caking and stickiness, are observed in these powders. To overcome these three related phenomena, some carriers such as maltodextrin are used to increase Tg and prevent powders from stickness during drying. Therefore, powders with a good physical stability are obtained in drying process (Bhandari & Hartel, 2005; Franceschinis, Salvatori, Sosa, & Schebor, 2014). Freeze drying process is widely used for pharmacological, biological materials, nanomaterials as well as foods. Seasonal fruits and vegetables are often dried for the purpose of providing their availability in the market for consumption all year round (Marques, Silveira, & Freire 2006; Ratti, 2001).

The concentration of the fruit juice is another preservation technique. It is a widely used method with less storage, packaging and transportation costs (Bozkir & Baysal, 2017). The long-shelf life product is obtained through concentration process by removing water from the fruit juice until desired °Brix is reached (Yaakob, 2012). The fruit juices are concentrated to approximately 65 °Brix by a suitable evaporation method (Ibarz et al., 2009). For this purpose, the thermal evaporation is the most common method used for the removal of water. Recovered and concentrated volatile compounds are also added back to the final concentrated juice product (Barbe, Bartley, Jacobs, & Johnson, 1998; Yaakob, 2012). However, thermal degradation of the nutrients and cooked flavour in the concentrated juice product is the disadvantages of the evaporation process. (Cassano & Drioli, 2007). In order to avoid overheating effects, vacuum is applied during the concentration process. Boiling point of the liquid is decreased and vapor transfer takes place more rapidly under vacuum conditions. This method also maintains the nutritional, color, flavor properties of the product (Bozkir & Baysal, 2017).

The main objective of this Ph.D. thesis was to manufacture different shelf stable products from unripe grapes (e.g. fresh verjuice, verjuice powder and concentrated verjuice) by using several food processing technologies. For this purpose, it was aimed to (i) produce and extend the shelf life of verjuice with minimum change in its "fresh like" properties by using the combination of the nonthermal UV-C irradiation or Pulsed-UV light (PUV) treatments assisted by mild heat, (ii) produce verjuice powder with a good storage stability by using freeze drying method, (iii) produce concentrated verjuice with minimal quality loss by using vacuum evaporation method.

This Ph.D. thesis covers 8 chapters. Chapter 2 includes the theoretical information about fruit juice including its composition, health value and microbiological safety.

Verjuice characteristics, nutritional value and health benefits were also reviewed in detail. The market potential of verjuice, verjuice powder and concentrated verjuice were also evaluated and discussed. Several fruit juice preservation methods, including conventional thermal pasteurization and alternative nonthermal technologies such as UV-C irradiation and Pulsed-UV light reviewed based on their inactivation mechanisms, industrial applications, advantages and disadvantages. The powder and concentrated form of verjuice obtained by freeze drying and vacuum evaporation methods were also discussed and explained in detail.

In chapter 3, suitable pasteurization conditions for verjuice was determined. 5-log reduction of target microorganism, i.e. *S. cerevisiae* (NRRL Y-139), in UV-C irradiation, mild heating and the combination of UV-C and mild heating processes. For this purpose, acid adapted *S. cerevisiae* strains were inoculated into freshly squeezed verjuice and exposed to UV-C light, mild heating and the combination of these treatment in a hurdle strategy. To assess the efficacy of the UV-C treatment, different initial loads of *S. cerevisiae* were studied in verjuice. The UV-C irradiation assisted with mild heating at different temperatures were applied to achieve 5D pasteurization of verjuice. Different kinetic models were applied to inactivation data of *S. cerevisiae* in the juice and the best fitted model was selected for the calculation of pasteurization time (5D) of verjuice. Synergism of the combined treatments were also determined in this chapter.

In chapter 4, microbiological, physicochemical and optical quality properties of verjuice pasteurized by UV-C irradiation assisted with the mild heating (the pasteurization conditions were determined from the chapter 3) were monitored during 12 weeks of refrigerated conditions. Untreated and thermally pasteurized verjuice samples were also evaluated as negative and positive controls during refrigerated shelf life.

Pasteurization conditions of verjuice using nonthermal pulsed-UV (PUV) technology were explored in chapter 5. In the first part of this chapter, different processing parameters of PUV, such as depth of juice layer, distance from the light and the number of the pulses, were evaluated based on the logarithmic reduction of the target microorganism in verjuice, i.e. *S. cerevisiae* (NRRL Y-139). The PUV conditions provided the highest microbial reduction was selected for the following experiments. In the following part of this chapter, the selected PUV conditions was combined with mild heating at different temperatures to achieve 5-log reduction of *S. cerevisiae* in verjuice. In the last part, several quality characteristics of verjuice pasteurized with the selected combined treatment conditions were monitored during 6 weeks of storage at refrigerated

and room temperatures. Untreated and thermally pasteurized juices were also analyzed and compared during shelf life. All experiments of the chapter 5 were carried out in the labs of Department of Food Technology, University of Lleida (UDL), Spain.

In chapter 6, shelf stable verjuice powder was produced by using freeze drying method. Maltodextrin concentration and freeze drying time were used as processing parameters. Verjuice feed mixtures were prepared by adding different concentrations of maltodextrin to freshly squeezed verjuice. These feed mixtures were freeze-dried at different drying times to obtain powders. Several quality properties of verjuice powders as well as yield of the drying process were analyzed and compared to determine the optimum drying conditions. Stability of the optimum verjuice powder were examined based on several quality properties during the accelerated storage conditions.

In Chapter 7, concentration conditions for verjuice were explored to obtain a product with a minimum quality loss. For this purpose, verjuice was concentrated up to a constant soluble solid content (65 °Brix) by using vacuum evaporation method operating at different temperatures/pressure combinations and conventional evaporation at atmospheric pressure (open-pan boiling). Some quality parameters of the concentrated juice samples were compared to determine the optimum evaporation conditions. Finally, the concentrated verjuice obtained under the selected evaporation conditions (based on several quality criteria) was evaluated during 12 weeks of storage at room temperature.

Chapter 8 is a conclusion chapter. The main findings obtained for the production of pasteurized verjuice, verjuice powder and concentrated verjuice were presented in a summary. Suggestions for the future works were also given in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Fruit juice is defined by Codex Alimentarius Commission as "the unfermented but fermentable liquid that is made from well, fully grown, fresh fruits or fruits harvested from suitable conditions". It is directly extracted from the fruit by feasible mechanical ways and has the physical, chemical, organoleptic and nutritional properties of fruits that are obtained from. Some of the fruit juice has seeds, pips or peels. Thus, the juice may be turbid or clear. It may be produced from single type or mixed fruits (Alimentarius, 2005).

According to the Turkish Food Legislation, fruit juice consists of 100% fruits, it is not reconstituted and not include additives (Cemeroglu, 2004). Fruit juice contains sugars, vitamins, minerals, organic acids, amino acids and bioactive compounds. Besides, it has many health promoting properties such as antioxidant, anti-inflammatory, anti-carcinogenic, anti-aging, cardiovascular disease and obesity lowering (Slavin & Lloyd, 2012; Zulueta, Esteve, Frasquet, & Frigola, 2007). Nowadays, the consumption of fruit juices has been rising because of their nutritional value and health benefits (Bates, Morris, & Crandall, 2001; Cemeroglu, 2004). Fruit juices and its products can be sold as unprocessed, processed, and fortified forms with vitamins, flavours and healthy compounds. They are stored in the refrigerators and freezers in the market (Barrett, Somogyi, & Ramaswamy, 2004). Nowadays, high-quality fresh-like juices are becoming more popular drink because they have been started to be produced using innovative approaches to preserve their original and nutritional quality.

In this chapter, the composition of fruit juice, its nutritional value and causes of its microbiological spoilage; several juice processing methods such as pasteurization, drying and concentration used for extending the shelf life of fruit juice products; and the general characteristics and potential use of verjuice, concentrated verjuice and verjuice powder are assessed.

2.2. Fruit Juice

There are various types of fruit juice and juice-like products defined by the Codex Alimentarius Commission sold in the market. The most common juice terms are listed in Table 2.1 with the brief definitions. The distinction and correct labelling of these juice products have a great importance for consumers. In this section, a detailed assessment of the composition, health benefits and safety of freshly squeezed fruit juice is carried out.

Table 2.1. Common juice definitions
(Source: Bates et al., 2001)

Term	Criteria	Remarks
Pure Juice 100%	All juice	No additional content, not concentrated
Fresh squeezed	Not pasteurized	Held refrigerated, Food safety concerns
Chilled, ready to serve	All juice	Held refrigerated, made from concentrate or pasteurized juice
Not from concentrate	Single strength	Pasteurized after extraction
From concentrate	Made from concentrate	Reconstituted and pasteurized
Fresh frozen	Unpasteurized	Single strength, frozen after extraction
Juice blend	All juice	A mixture of pure juice
Puree	Pulp-containing	More viscous than juice, totally fruit
Nectar	Pulpy or clear	Sugar, water, acid added, 25-50% juice
Nectar base	Requires reconstitution	Possess sufficient after flavour, acid and sugar to require water dilution for consumption
Juice drink	Low in juice	Contains 10% to 20% juice
Juice beverage	Low in juice	Contains 10% to 20% juice
Juice coctail	Low in juice	Contains 10% to 20% juice
Fruit + "ade" (suffix)	Lemonade	Contains >10% juice, sugar and water
Juice extract	Water extract	Fruit extracted by water, and then concentrated
Fruit punch	Token juice	1% juice + natural flavors
Natural flavoured	Token juice	Usually >1% juice

*: different country standards for juice solids minimum

2.2.1. Nutritional Value and Composition of Fruits and Fruit Juices

It is well known that fruits are the essential source of the vitamins, minerals and bioactive compounds. Therefore, daily fruit consumption is recommended to provide some health benefits (Liu et al., 2000; Saikia, Mahnot, & Mahanta, 2014). The composition of the fruit juice is similar to the fruit that is obtained from. The general composition of the edible part of fruits was listed in Table 2.2.

Table 2.2. Composition of edible part of fruits (fresh wb)
(Source: Bates et al., 2001)

Component	Range (%)	Commends
Water	97 - 70	Influenced by cultivation and post-harvest conditions
Carbohydrates	25 - 3	Sugars and polymers - pectin, hemicellulose, cellulose
Protein	5 - trace	More in oily fruit and seeds
Lipids	25 - trace	Traces in cell membrane, in seeds, high in avocado
Acids	3 - trace	Citric, tartaric, malic, lactic, acetic, ascorbic + minor
Phenolics	0.5 - trace	Tannins and complex phenols
Vitamins	0.2 - trace	Water soluble > fat soluble
Minerals	0.2 - trace	Soil and species dependent
Diatery fibre	<1 to >15	Peel and core dependent
Pigments	0.1 - trace	Carotenoids, anthocyanins, chlorophyll

The main ingredient of the fruit juice is water. Most of the fruits contain water at various concentrations changes between 70% (over ripe grapes) and 97% (some wild berries) depending on the fruit, maturity and agricultural variability (Bates et al., 2001). The water is distributed along the edible part of the whole fruit without skin (Marques et al., 2006). Thus, water soluble components such as sugars, acids, free amino acids, minerals, water soluble vitamins and phenolic matter are expected to be transferred to the juice. However, water-insoluble materials such as polysaccharides (cellulose and pectic matters), lipids and carotenoids are not transferred to the (clear) juice (Cemeroglu, 2004).

The second component of the fruit is carbohydrates that form the main solid part of the fruit juice. Fruits are very rich in two kinds of carbohydrates; water soluble sugars (glucose, fructose, saccharose and mannose) that are directly transferred to the juice and insoluble polysaccharides (pectin, cellulose, hemicellulose) that are the residue after pressing of the fruit (Bates et al., 2001). Therefore, fruit juices contain glucose and fructose as well as a little amount of saccharose and mannose. Although fructose and glucose are commonly found in fruit juices, saccharose content varies depending on the types of fruits (Artik & Velioglu, 1992; Cemeroglu, 2004).

Other than water and carbohydrates, fruits are also rich in vitamins such as βeta-carotene (pro-vitamin A) and ascorbic acid (vitamin C) that are heat sensitive nutrients (McBean, Joslyn, & Nury, 1971). Vitamin A, C, E that are the essential vitamins found in fruit juice have antioxidant properties supporting cardiovascular health, and providing anti-carcinogenic, anti-aging and protective effect on Alzheimer disease (Zulueta et al., 2007). Fruit juice containing pro-vitamin A carotenoids has antioxidant activity, as well

(Gardner, White, McPhail, & Duthie, 2000; Pena, Salvia-Trujillo, Rojas-Grau, & Martin-Belloso, 2011).

Organic acids have an important role in the plant metabolism, spoilage and adulteration and used as preservative in the fruit juices (Soyer, Koca, & Karadeniz, 2003). These acids are responsible for sour taste and low pH of some fruit juices (Bates et al., 2001). Non-volatile organic acids such as tartaric, citric and malic acids affect flavour, colour and stability of fruit juices. Furthermore, volatile acids such as formic and acetic acid increase mashing performance of the juices (Cunha, Fernandes, Faria, Ferreira, & Ferreiara, 2002). Essential minerals that are obligatory for human growth and health such as potassium, magnesium, calcium, sodium, zinc, copper and iron are the main minerals in fruit juice (Winiarska-Mieczan & Nowak, 2008).

Fruits are not considered as a protein and fat source (Cemeroglu, 2004). Proteins and lipids generally present in fruits are less than 1 and 0.5%, respectively, except seeds and oily fruits. Thus, they cannot be fully transferred to the juice. However, free amino acids are transferred to the juice during processing. Fruit juices have a low calorie value due to the presence of trace amount of lipids in their compositions. Thus, juices play an essential role in the fat-free diets. Additionally, they are “heart healthy” because of the fatty acids profile and fat-soluble vitamins (Artik & Velioglu, 1992; Bates et al., 2001).

Fruits and fruit juices includes many types of phytochemicals having health promoting benefits. Flavonoids (anthocyanins, catechins, flavones, quercetin, resveratrol, etc.) are important phenolic elements in fruit juices and have many health benefits such as being antioxidant, impressing blood circulation, providing more conductance and flexibility of the capillaries (Ashurst, 2005). Carotenoids (provitamin A), chlorophyll, vitamin C (ascorbic acid), vitamin E (tocopherol), phenolic acids (cafeic, gallic, ellagic, capsaicin, carnisol etc.) are the other phytochemicals playing a crucial role against cancer and cardiovascular disease (Bates et al., 2001; Broihier, 1999; Guhr & LaChance, 1997).

2.2.2. Microbial Spoilage of Fruit Juice and Regulatory Issues

Fruits are high-value plant materials but they are perishable. They spoil easily and loss their important nutrients immediately after harvesting due to improper post-harvest methods, unsuitable transportation conditions, storage and processing (Marques et al., 2006). Untreated fruit juice is more sensitive to quality loss and spoilage than whole fruits

because its components more in contact with air and more accessible for microorganisms. Table 2.3 shows major deteriorations in fruit juices. Main quality losses in fruit juices may occur as a consequence of contamination by pathogen and spoilage microorganisms.

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

Spoilage and pathogenic microorganisms can directly infect and grow in the highly acidic and nutritious environment of fruit juices, and cause many quality defects, foodborne illnesses and outbreaks (Tournas, Heeres, & Burgess, 2006). Considering these microorganisms, pathogens are the most important ones affecting the human health and causing different foodborne outbreaks. However, the limited number of pathogenic bacteria (aciduric bacteria) can survive in highly acidic foods and result in several illnesses after their consumption (Table 2.4). On the other hand, lactic acid bacteria, yeasts and molds, which are the predominant microorganisms in the low-pH foods, are the main microorganisms responsible from the deteriorations of fruit juices (Barrett et al., 2004). It is known that *Lactobacillus*, *Leuconostoc*, *Acetobacter*, *Glucanobacter* spp. are the well-known lactic acid bacteria species causing juice deterioration. Besides, yeasts such as *Pichia, Candida, Saccharomyces, Zygasaccharomyces,* and *Rhodotorula* are commonly encountered genera responsible from the spoilage of juices. Moulds such as *Penicillium* sp., *Aspergillus* sp., *Eurotium*, *Alternaria*, *Cladosporium*, *Paecilomyces*, and *Botrytis* have also been reported in juice spoilage (Aneja, Dhiman, Aggarwal, Kumar, & Kaur, 2014; Casolari, 1989).

Table 2.4. Growth conditions of foodborne pathogenic bacteria
(Source: Jongen, 2005)

Pathogens	**Temperature (°C)**	**pH**	**a_w**
Salmonella spp.	6.5-47	4.5-?	>0.95
Clostridium Botunilum (A&B)	10-50	4.7-9	>0.93
Staphylococcus aureus	7-45	4.2-9.3	>0.86
Escherichia coli	2.5-45	4.6-9.5	>0.935
Listeria monocytogenes	0-45	4.4-9.4	>0.92
Yersinia enterocolitica	1-44	4.4-9	NR
Bacillus cereus	10-49	4.9-9.3	>0.95
Campylobacter jejuni	25-42	5.5-8	NR
Vibrio parahaemolyticus	12.8-40	5-9.6	>0.94
Clostridium perfringens	10-52	5.5-8	>0.93
Streptococcus pyogenes	>10-<45	4.8- <9.2	NR

The microbial spoilage of foods can be detected by visually (slime, colonies, color degradation). It may also deteriorate the texture (destruction of polymeric compounds, cloud loss), and cause undesirable flavour and odours (Aneja et al., 2014; Gram, Trolle, & Huss, 1987). Spoilage occurs depending on the type of microorganisms and compositions of the fruit juice. For example, lactic acid bacteria utilize carbohydrates and produce several compounds i.e., lactate, ethanol, alcohol, acetic acid, acetate, CO_2, diacetyl and acetoin that are responsible from off-flavor and off-odor. 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, glycerol, acetaldehyde, pyruvic acid, and α-ketoglutaric acid. Moulds can grow in the presence of dissolved oxygen and cause off-flavor, color degradation as a result of some enzymatic activities and produce toxins in the juice as a result of some enzyme activities (Filtenborg, Frisvad, & Thrane, 1996; Rankine, 1968; Ray, 2004; Tournas et al., 2006). Therefore, the contamination and growth of these microorganisms into the fruit juices must be controlled by applying certain food preservation techniques. Fruit juice producers must comply with regulation in food legislation and apply control measures (Bates et al., 2001; Ray, 2004; Vantarakis, Affifi, Kokkinos, Tsibouxi, & Papapetropoulou, 2011).

All production steps in juice manufacturing starting from field until reaching to the table, i.e. steps during the cultivation, harvesting, transportation, storage, processing, packaging, distribution and marketing, should be monitored in order to produce safe fruit juice. For this reason, several regulatory programmes such as on-Farm Food Safety

(OFFS), Good Agricultural Practice (GAPs), Good Manufacturing Practices (GMPs) and Hazard Analysis and Critical Control Point (HACCP) are being applied to reduce the safety risks in the juice industry. These procedures are based on two principles; to provide safety for all components of the product and preventing adulteration in the manufacturing (Bates et al., 2001). OFFS programme is used by fruit producers to reduce the risks during fresh fruit cultivation but it has some difficulties on the verified information, financial resources, suitable documentation (Jongen, 2005). Among these regulatory programmes, HACCP management systems implemented by the United States Food and Drug Administration (U.S. FDA) are commonly used in all production steps of fruit juice. These systems can inhibit outbreaks caused by pathogens and food deterioration occurred by spoilage microorganisms. HACCP is defined as a systematic approach based on the evaluation of hazards, determination of critical control points, controlling for each production steps from fruit producers to juice consumers by International Fresh-cut Produce Association [IFPA], Western Growers Association [WGA] and United Fresh Fruit & Vegetable Association. This regulation is also recommended by US National Academy of Sciences, World Health Organization, Codex Alimentarius Committee and U.S. FDA. According to the rule of this regulation (21 CFR 110), the 5-log reduction must be targeted to the "pertinent pathogen." in the juice. The "pertinent pathogen" is the most resistant microorganism of public health concern that may occur in the juice. Thus, 5D pasteurization is required to be employed to produce safe fruit juice (U.S. FDA, 2001).

2.3. Preservation Methods of Fruit Juices

Food preservation is a continuous process used to avoid deterioration of the foods by enzymes and spoilage microorganisms and to protect the foods from the pathogenic microorganisms causing foodborne illnesses. Fruit juice is a highly perishable food with a short shelf life. Besides, some of the juices are seasonal or regional, therefore, their consumption can be possible in a certain period of the year or place of the world (Barrett & Lloyd, 2012; Devlieghere, Vermeiren, & Debevere, 2004). For these reasons, several traditional preservation methods (high-temperature pasteurization, chemical preservation, acidification etc.) are used in the fruit juice processing. Besides, new emerging and novel nonthermal technologies (high hydrostatic pressure, pulsed electric field, ultrasound, UV-C irradiations, pulsed-UV light, etc.) have been developed (Butz &

Tauscher, 2002). The important aims of these preservation technologies are to extend the shelf life of juice by destroying the pathogen and spoilage microorganisms, to prevent the enzymatic activities, to maintain the organoleptic and nutritional quality, to leave no residue after processing, to have low cost, to be used practically, to have no negative effect on consumers (Raso & Barbosa-Canovas, 2003).

2.3.1. Thermal Pasteurization

Conventional thermal pasteurization is known as the most common preservation method for the fruit juices (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007; Azhu Valappil, Fan, Zhang, & Rouseff, 2009; Tiwari, O'Donnella, & Cullen, 2009a). The main objective of thermal pasteurization is to kill all vegetative pathogen microorganisms and partial inactivation of the spoilage microorganisms such as yeasts, moulds, bacteria and viruses as well as the enzymes. Juice having pH 4.5 or less is exposed to pasteurization at different temperature ranges between 60 and 100 °C for a few seconds (Rivas et al., 2006). There are different types of thermal processing methods for fruit juice pasteurization including HTLT (High temperature long time known as conventional pasteurization, HTST (high temperature short time), MTLT (mild temperature long time) and MTST (Mild temperature short time) treatments (Petruzzi et al., 2017). The processing conditions and the main quality effects of these thermal treatments are listed in Table 2.5.

Table 2.5. Thermal processing for fruit juice pasteurization
(Source: Petruzzi et al., 2017)

Treatment	Processing conditions	The main effect on the juice quality
HTLT	≥80°C, >30s (conventional pasteurization)	Undesirable sensorial, nutritive and bioactive quality properties
HTST	≥80°C, ≤30s	Maintain bioactive compounds Sensorial changes (aroma, flavour, color, texture)
MTLT	<80°C, >30s	Improve shelf life of minimally processed products Reduction of vitamins, antioxidant property
MTST	<80°C, ≤30s	Limited effect on the sensorial quality Physicochemical, functional and color degradation

Food and Drug Administration (FDA) is defined the pasteurization as "the required temperature and time combination for 5-log reduction of the most resistant microorganisms" (U.S. FDA, 2001). Recently, some alternative processing technologies have been emerged and this definition is extended by the USDA National Advisory Committee on Microbiological Criteria for Foods (NACMCF) as "Any process, treatment, or combination thereof that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage" (NACMCF, 2006). The target microorganism for pasteurization can be the heat resistant pathogens such as *E. coli* O157:H7 and *Listeria monocytogenes* or spoilage microorganisms such as yeasts and moulds. Miller and Silva (2012) defined the typical pasteurization conditions for fruit juices as 65 °C for 30 min, 77 °C for 1 min, or 88 °C for 15 s. Generally, juices are treated at 90 °C for 1 min to extend the shelf life of the juice (Timmermans et al., 2011). For example, grape juice is commercially pasteurized at 90 - 95 °C for 15 - 30 s, however, apple or orange juice are pasteurized at 96 °C for 15 - 30 s and 90°C for 60 s, respectively (Cemeroglu, 2004). Several pasteurization studies were reported in the literature and significant reductions were achieved by inactivating target microorganisms in the fruit juices (Elez-Martinez, Escola-Hernandez, Soliva-Fortuny, Martin-Belloso, 2005; Gabriel & Nakano, 2009; Wu, Mittal, & Griffiths, 2005). 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 (Charles-Rodriguez, Nevarez-Moorillon, Zhang, Ortega-Rivas, 2007; Garde-Cerdan et al., 2007; Walkling-Ribeiro et al., 2008). A summary of some important studies related to the quality degradations by thermal processes are listed in Table 2.6.

2.3.2. Nonthermal Preservation Techniques

Generally, the "nonthermal" term are used for the intensity of temperature that are non-hazardous for foods. In recent years, consumers demand more healthy, freshly-like, and nutritive foods. Therefore, the nonthermal technologies are develop to minimize quality damages caused by thermal pasteurization including degradations of nutrients, off-color and off-flavour of the products.

Table 2.6. The effect of thermal processing treatments on the fruit juice quality (Source: Petruzzi et al., 2017)

Juice	**Processing Conditions**	**Findings**	**Reference**
Aonla, bottle gourd, ginger, lemon juice blend	HTLT 80 to 95 / 5 to 30 min	Minimum and maximum loss of ascorbic acid of juice blend were 22.97% at 80 °C for 5 min and 47.70% at 95 °C for 30 min, respectively	Gajera and Joshi (2014)
Blackberry juice	HTLT 80 and 90 °C / 0 to 300 min	The antioxidant activity of juice was reduced as a result of temperature increase.	Zhang et al. (2012)
Cranberry nectar	HTLT 80 °C /2 min	Significant decrease in the consistency coefficient	Simunek et al. (2014)
Peach Juice	HTLT 90 °C / 5 min	Significant reductions in total carotenoids, protocatechuic acid, zexanthin and β-cryptoxanthin	Oliveira et al. (2012)
Red raspberry Juice	HTLT 80 °C/15 min	The content of the ascorbic acid was reduced by 47% and 31% in fresh and processed juice after 20 day of refrigerated storage	Yang et al. (2015)
Black mulberry Juice	HTST 90 °C/30 s	Reduction of the antioxidant activity	Jiang et al. (2015)
Grapefruit Juice	HTST 80 °C/11 s	Significant decrease in citric and ascorbic acids	Igual et al. (2010)
Lemon Juice	HTST 90 °C/15 s	Decrease of total carotenoid content	Ucan et al. (2016)
Watermelon Juice	HTST 90 °C/30 s	Low viscosity values over the subsequent refrigerated storage	Aguilo-Aguayo et al. (2010)
Apple, banana, orange, strawberry Smoothie	MTLT 70 °C/10 min	Reduction of the total antioxidant capacity, total phenols, anthocyanins and color	Keenan et al. (2012)
Pineapple Juice	MTLT 75 °C/3 min	Decrease in total flavonoid content	Saikia et al. (2015)
Carrot, orange Juice blend	MTST 72 °C/26 s	Negative effects on color and flavor	Caminiti et al. (2012)

Nonthermal technologies are resulted in production of high quality foods. They are also environmentally friendly, low cost, more economic methods which providing additional value in the foods when comparing to conventional thermal pasteurization method (Artes & Allende, 2005; Pereira & Vincete, 2010).

The general principle of the nonthermal preservation is "to process as little as possible, as much as necessary". These alternative methods provide an effective inactivation of microorganisms and enzymes without changing the nutritional and sensorial quality of the foods comparing to thermal pasteurization (Butz & Tauscher, 2002). FDA also aimed for 5-log reduction of the most resistant pathogen using nonthermal technologies for pasteurization (U.S. FDA, 2001). In order to obtain minimally processed foods as well as to meet FDA requirements for pasteurization, these technologies can also be used in a combination (hurdle strategy). The general advantages and disadvantages of the minimally processed foods are summarized in Table 2.7. These alternative nonthermal processing methods for juices include pulse electric field (PEF), high hydrostatic pressure (HHP), ultrasound, ultraviolet (UV-C) irradiation and pulsed-UV (PUV) light. Among these technologies, UV-C irradiation and Pulsed-UV light are the pasteurization methods based on the exposure of fruit juices to light. They are explained in detail in the forthcoming sections of this chapter.

Table 2.7. The 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 manufacturer	
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

2.3.2.1. Ultraviolet Light (UV-C) Irradiation

Ultraviolet (UV) light covers the wavelength region from 100 to 400 nm in an electromagnetic spectrum (Figure 2.1) and it is divided into four subgroups (Barbosa-Canovas, Tapia, & Cano, 2005b). UV-A or long wave UV light (315 to 400 nm) is responsible for tanning of skin; UV-B or medium wave UV light (280-315 nm) causes skin burns and skin cancer; UV-C or short wave UV light (200-280 nm) that is in the germicidal spectrum results in destroying of microorganisms. Vacuum UV range that is in the range of 100 and 200 nm is absorbed by all materials, transmission of light can be achieved with only vacuum conditions (Karel & Lund, 2003; Koutchma et al., 2009).

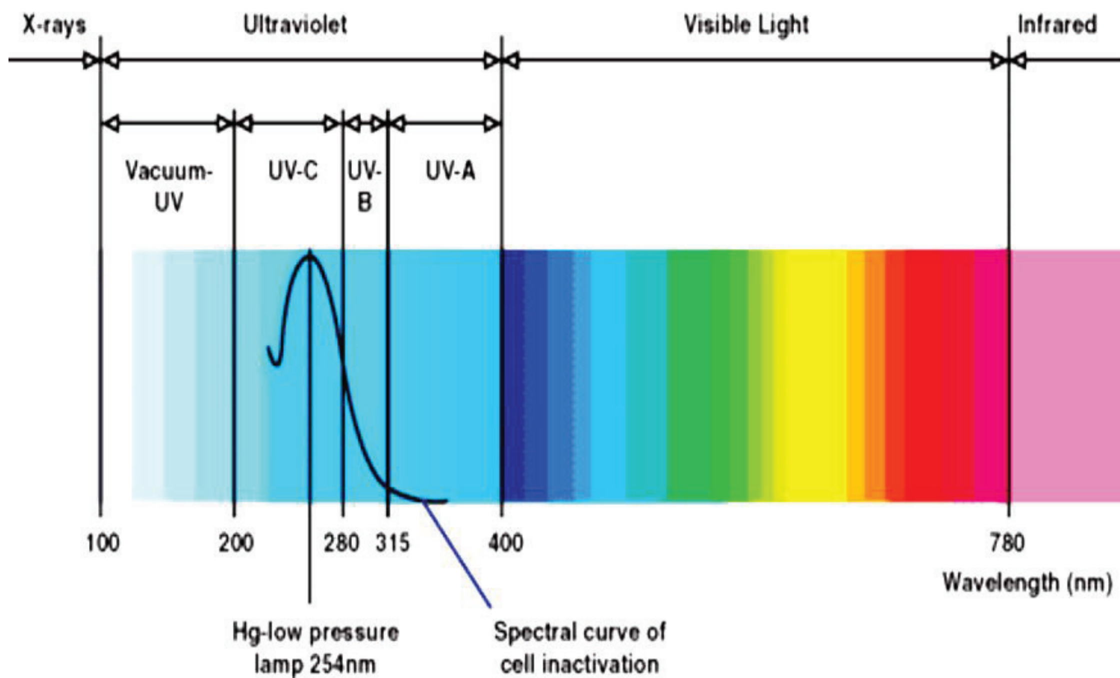


Figure 2.1. The electromagnetic spectrum
(Source: Aqualight, 2018)

UV-C light at around 254 nm is the most influential wavelength since the light absorbed by DNA of microorganisms at this wavelength is at the highest level (Green, Basaran, & Swanson, 2003; Koutchma et al., 2009). The peak point as 253.7 nm prevents transcription and reproduction of the cells by passing from their cell wall and forming dimerization on thymine structure in their DNA (Figure 2.2). DNA forms by bonding of double stranded bases, i.e. pyrimidine bases (thymine, cytosine) in one strand and purine

bases (adenine, guanine) in the other strand. Hydrogen bonding presents between strands of DNA, thus, adenine-thymine and guanine-cytosine base combinations occur. In DNA of the cell, hydrogen bonding between the pyrimidine bases is ruptured by the effect of UV-C light and adjacent thymine molecules are bounded as pyrimidine dimers. Thus, transcription and reproduction of the cell is inhibited and cell death is accelerated (Bank, Schmehl, & Dratch, 1990; Bintsis et al., 2000; Miller, Jeffrey, Mitchell, & Elasri, 1999). Efficiency of the UV irradiation depends on the sensitivity of the different microorganisms to UV-C light. Several factors that are cell wall structure, thickness and composition, availability of UV absorbing proteins and varieties in the nucleic acid configuration have an effect on their sensivities (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 et al., 2005b). Johnson, Anil Kumar, Ponmurugan, and Gananamangai (2010) indicated that the smallest cells are the most resistant to UV light due to the amount of UV absorbed per cell. However, yeasts and moulds are also more resistant to UV light, because they have less pyrimidine bases and have different cell membrane and thickness (Tran & Farid, 2004). The factors affecting to UV-C irradiation can be listed as variability of the species, number of microorganisms, stage of microorganisms, irradiation conditions and UV-C source (Barbosa-Canovas et al., 2005b). 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).

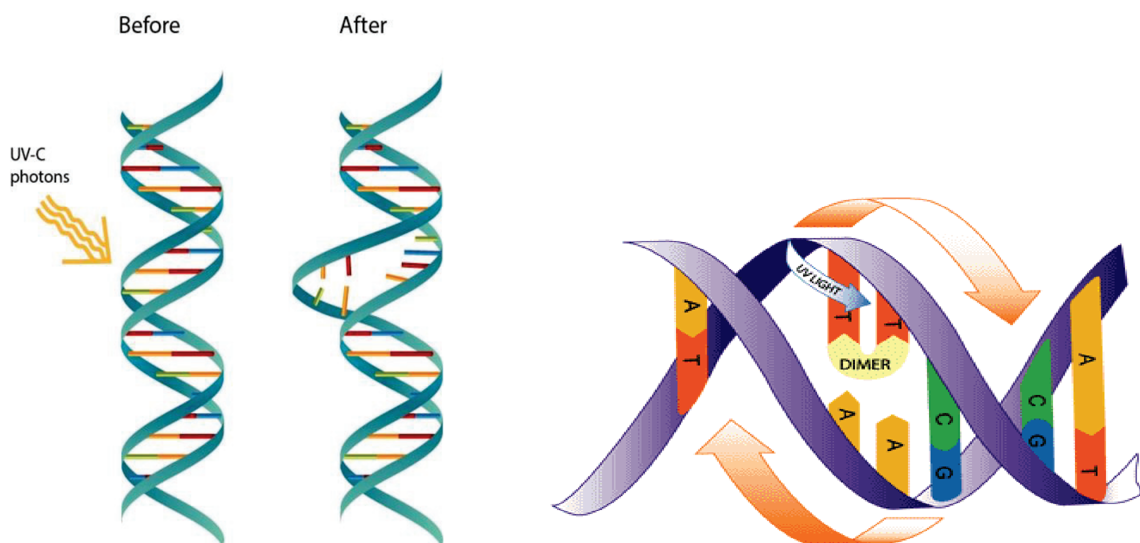


Figure 2.2. DNA structure after UV-C irradiation

Low pressure mercury lamps produce 253,7 nm monochromatic UV light are mainly used for the inactivation systems due to low operation temperature and power requirements (Jonhson et al., 2010). The main advantages of UV-C irradiation are listed as environmentally friendly, easy to set up, no influence on flavour, color and odour of foods, safe process, no residual present after process and compatible with other technologies (Bintsis et al., 2000; Johnson et al., 2010; Pereira & Vicente, 2010). However, the application of UV-C irradiation is restricted for certain fruit juices due to the presence of high amount of color compounds and soluble and/or suspended particles, which reduce the penetration capacity of UV light (Koutchma, Keller, Chirtel, & Parisi, 2004). Generally, UV-C irradiation is 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 of liquid foods and beverages (Begum, Hocking, & Miskelly, 2009; Koutchma, 2008; Koutchma et al., 2009). Recent studies showed that UV-C irradiation is successively used for fruit juice and milk pasteurization in food industry (Caminiti et al., 2012b; Gachovska, Kumar, Thippareddi, Subbiah, & Williams, 2008; Koutchma et al., 2004; Matak et al., 2005) in addition to air disinfection, pathogen inactivation in meat, poultry and fish products (Wong, Linton, & Gerrard, 1998; Liltved & Landfald, 2000).

Considering UV-C pasteurization of fruit juice, several studies have been conducted to evaluate microbial efficacy in the many types of fruit juices (Abdul Karim Shah, Shamsudin, Abdul Rahman, & Adzahan, 2016). Noci et al., 2008 obtained 2.2 log reductions in the number of total aerobic counts in apple juice after UV-C irradiation for 30 min by using a batch system with a 30 W UV-C light. However, Caminiti et al. (2012b) achieved more than 4 log CFU/mL reduction in *E. coli* and *L. innocua* in apple juice after a UV dose of 2.66 J/cm^2 in the annular continuous flow UV system. Kaya, Yildiz & Unluturk (2015) found more than 6 log reductions in *E. coli* K-12 strains inoculated into the Lemon Melon Juice Blend with 2.46 J/cm^2 UV dose by using continuous flow UV system. Unluturk and Atilgan (2014) found that the inactivation of *E. coli* K-12, lactic acid bacteria and foodborne yeasts were 3.8, 4.1, 1.6 log CFU/mL, respectively, after 116.7 J/mL UV dose in the same UV system. Pala and Toklucu (2013b) achieved 5.72 log reduction of *E. coli* ATTC 25922 in orange juice treated with a UV dose of 36.09 kJ/L. Several studies related to UV-C irradiation for pasteurization of fruit juices are summarized in Table 2.8.

Table 2.8. UV-C irradiation treatment of fruit juices
(Source: Abdul Karim Shah et al., 2016)

Fruit juice	**UV-C dosage**	**Target microorganism**	**Log reduction**	**Reference**
Pineapple juice	0.154 mL/s	*S. typhimurium*	3.0	Mansor et al. (2014)
Lemon-melon juice blend	0.44–2.86 mJ/cm^2	*E. coli* K-12	0.06 – 6	Kaya et al. (2015)
Apple cider	14.0 mJ/cm^2	E. coli O157:H7	7.2	Basaran et al. (2004)
Apple juice	7.7 kJ/L 9.6 kJ/L 3.9 kJ/L 9.6 kJ/L	*E. coli* *S. cerevisiae* *L. plantarum* *A. acidoterrestris*	6.0 4.0 >5.0 4.0	Muller et al. (2011)
White Grape juice	138 mJ/cm^2 280 mJ/cm^2 180 mJ/cm^2	*S. cerevisiae* Yeasts LAB	5.0 3.0 4.3	Kaya and Unluturk (2016)
Pomegranate juice	62.4 J/mL 62.4 J/mL 62.4 J/mL	*E. coli* Aerobic plate count Yeasts and molds	6.2 1.8 1.5	Pala and Toklucu (2011)
Water melon juice	2.7 - 37.5 J/mL	Aerobic plate count	1.5	Feng et al. (2013)
Guava juice Pineapple juice	918 J/L 1377 J/L	Yeasts and molds Aerobic plate count	4.5 3.3	Keyser et al. (2008)
Red grape juice	3672 J/L 3672 J/L 3672 J/L	*B. bruxellensis* *S. cerevisiae* *L. plantarum*	>2.0 >5.0 >5.0	Fredericks et al. (2011)
Orange juice	36.1 kJ/L	*E. coli O157:H7*	5.7	Pala and Toklucu (2013b)

2.3.2.2. Pulsed-UV (PUV) Light Technology

Pulsed-UV Light (PUV) technology is used to kill pathogen and spoilage microorganisms in foods and to maintain food quality by keeping nutritional loss at minimum level. In this method, decontamination of foods is ensured by using short time high-peak pulses of broad spectrum wavelengths between 200 nm - 1 mm (Dunn et al., 1995; Oms-Oliu et al., 2010b). Foodstuff is exposed at least of one pulse of light with an energy intensity of 0.01-50 J/cm^2, and 1-20 flashes per second ranging from 1μs to 0.1s (Barbosa-Canovas, Pothakamury, Palou, & Swanson, 1998). Xenon lamps (200-1000 nm) approved by the FDA are used in PUV technology. Ultraviolet-C (UV-C) region of these lamps have great importance for the inactivation of microorganisms (Gomez-Lopez et al., 2007; Koutchma, 2008). Microbial inactivation occurs by different mechanisms such as the breakdown of their DNA chain (photochemical effect); and the heating of the cell and chemical changes in protein, membrane and cell wall of microorganisms (photothermal and photophysical effect). The cell can be recovered itself in traditional or continuous UV treatments, however in PL technology, the cell is irreversibly damaged upon application of high energy and UV intensity and it cannot repair itself. For example, the equal quantities of microbial reduction can be achieved six times faster by Pulse UV light than in continuous UV light system (Fine & Gervais, 2004; U.S. FDA, 2000).

Studies about the PUV light indicate that this technology can be promising alternative method for the surface disinfection of the several solid foods such as meat products, vegetables and fruits. Dunn et al. (1988) achieved 1.5 logarithmic reductions in the number of *Pseudomanas* species by 16 J/cm^2 intensity applying two pulses with 0.5 ms intervals. In another study on a fish product, coliforms and psychrotrophs were reduced by 3 log with the combination of PUV and high pressure treatments (Dunn et al., 1988). In raw eggs, a reduction of 8 log units was achieved for *Salmonella enteriditis* after 0.5 J/cm^2 of pulse treatment (U.S. FDA, 2011). Ramos-Villarroel, Martin-Belloso, & Soliva-Fortuny (2011) reported a decrease of 2.97 and 3.33 log CFU/g in the fresh cut avocado inoculated with *Listeria innocua* and *Esherichia coli* respectively after the treatment of 30 pulses and intensity of 0.4 J/cm^2. Bialka & Demirci (2008) performed 3.9 log CFU/g reduction of *E. coli* O157:H7 in raspberry samples by applying 72 J/cm^2 Pulse UV treatment.

Recently, PUV technology tried to be used in pasteurization of liquid foods such as fruit juices. For example, Pataro et al. (2011) studied *L. innocua* ve *E. coli* DH5-α inactivation in apple and orange juices by using continuous flow PUV system. They reported 4.0 and 2.9 log CFU/mL reduction of *E. coli* and 2.98 and 0.3 log CFU/mL reduction of *L.innocua,* respectively, in apple and orange juice, after the treatment of 4 J/cm^2 PUV dose with a flow rate of 17 mL/min. Ferrario, Alzamora, and Guerrero (2015) investigated the effect of PUV on the inactivation *S.cerevisiae* cells in commercially pasteurized (clear) and freshly squeezed (cloudy) apple juice samples. Although *S.cerevisiae* cells in cloudy apple juice were inactivated as 2 log CFU/mL after 71.6 J/cm^2 PUV dose, this reduction was increased up to 4 log CFU/mL at the same dosage level in the clear juice. Maftei, Ramos-Villarroel, Nicolau, Martin-Belloso, and Soliva-Fortuny (2014) studied the inactivation of *Penicillium expansum* in clear apple juice by using different processing conditions. They obtained more reductions in apple juice samples having less thickness and less initial microbial load. *Penicillium* molds could be reduced up to 3.76 log with a total dose of 32 J/cm^2 after 40 pulses. Several studies related to PUV technology for pasteurization of fruit juices are summarized in Table 2.9.

2.3.2.3.Hurdle technology

Hurdle technology is called as combined methods, combined processes, combination preservation or barrier technology in literature (Leistner & Gorris, 1995). This strategy, which involves the application of a combination of emerging preservation treatments or conventional treatments at mild conditions, has become popular in recent years. Hurdle technology can be defined as "deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors (hurdles), to provide microbiological stability and preserve the sensory quality of foods as well as their nutritional and economic properties" (Leistner & Gorris, 1995; Leistner, 2000). The most common hurdles used in the preservation of foods are temperature, pH, water activity, chemical preservatives (nitrite, nitrate etc.), competitive microorganisms (LAB). In last decades, novel minimal processing technologies have begun to be used as hurdles. The main target of hurdle technology is to obtain microbiologically stable food products by combining the mild effect of these technologies (Abdul Karim Shah et al., 2016; Leistner & Gorris, 1995; Ross, Griffiths, Mittal, & Deeth, 2003).

Table 2.9. Pulsed-UV light processing of fruit juices
(Source: Bhavya & Umesh Hebbbar, 2017; Kramer, Wunderlich, & Muranyi, 2017)

Fruit juice	**PUV fluence**	**Target microorganism**	**Log reduction**	**Reference**
Apple juice	12.6 J/cm^2	*E. coli* ATCC 25922 *E. coli* O157: H7	2.66 2.52	Sauer and Moraru (2009)
Apple juice	4 J/cm^2	*E. coli* *L. innocua*	4.0 2.98	Pataro et al. (2011)
Orange juice	6 J/cm^2	*E. coli* *L. innocua*	2.02 1.77	Pataro et al. (2011)
Orange Strawberry juices Apple juice	71.6 J/cm^2	*L. innocua* *E. coli* *S. enteritidis* *S. cerevisiae*	0.3-0.8 (orange and strawberry) 1.6 (apple) 0.3-0.8 (orange and strawberry) 2.1 (apple)	Ferrario et al. (2015)
Apple juice Orange juice Grape juice Plum juice	0 - 29.21 J/cm^2	*P. aeruginosa* ATCC 10145	>7.0 (plum and apple) Up to 2.0 (grape) Up to 1.0 (orange)	Hwang et al. (2015)
Orange juice	4.3 - 5.1 J/cm^2	*E. coli* DSM 1607	2.42	Munoz et al. (2011)
Apple juice Melon juice Orange juice Strawberry juice	2.4 - 71.6 J/cm^2	*L. innocua* ATCC 33090	Up to ~4_5 log (apple juice) Up to ~1 log (orange juice) Up to ~6 log (melon juice) Up to ~0_2 log (strawberry juice)	Ferrario et al. (2013)
Apple juice	2.0 - 32 J/cm^2	*P. expansum*	1.2 - 3.76	Maftei et al. (2014)

Hurdle technology used for microbiological stability of foods is based on the synergistic or additive effect of the combination of each treatment at low individual intensities (Figure 2.3). Synergism means that the inactivation effect of the combined treatment was more than the inactivation effect of each treatment individually. Otherwise, the hurdle process would be additive (the sum of the effects of treatments). Synergistic effect is more preferable when using a hurdle technology (Raso & Barbosa-Canovas, 2003). Many studies have been conducted with fruit juices using combination of nonthermal technologies such as pulse light and ultrasound (Ferrario et al., 2015); ultrasound and high hydrostatic pressure (Abid et al., 2014); UV-C irradiation and pulse electric field (PEF) (Noci et al., 2008); PEF and high intensity light pulses (HILP) (Caminiti et al., 2011a). These technologies can also be applied at mild heat temperature in a hurdle strategy. There are many studies in the literature investigating the inactivation efficacy of UV-C irradiation and Pulsed-UV light methods in fruit juices which assisted with mild heating (Carrillo, Ferrario, & Guerrero, 2017; Gayan, Manas, Alvarez, & Condon, 2013; Gayan, Serrano, Alvarez, & Condon, 2016; Gayan, Serrano, Monfort, Alvarez, & Condon, 2012; Gayan, Torres, Alvarez, & Condon, 2014; Gouma, Alvarez, Condon, & Gayan, 2015a; Gouma, Gayan, Raso, Condon, & Alvarez, 2015b; Hilton, Moraes, & Moraru, 2017; Marquenie et al., 2003).

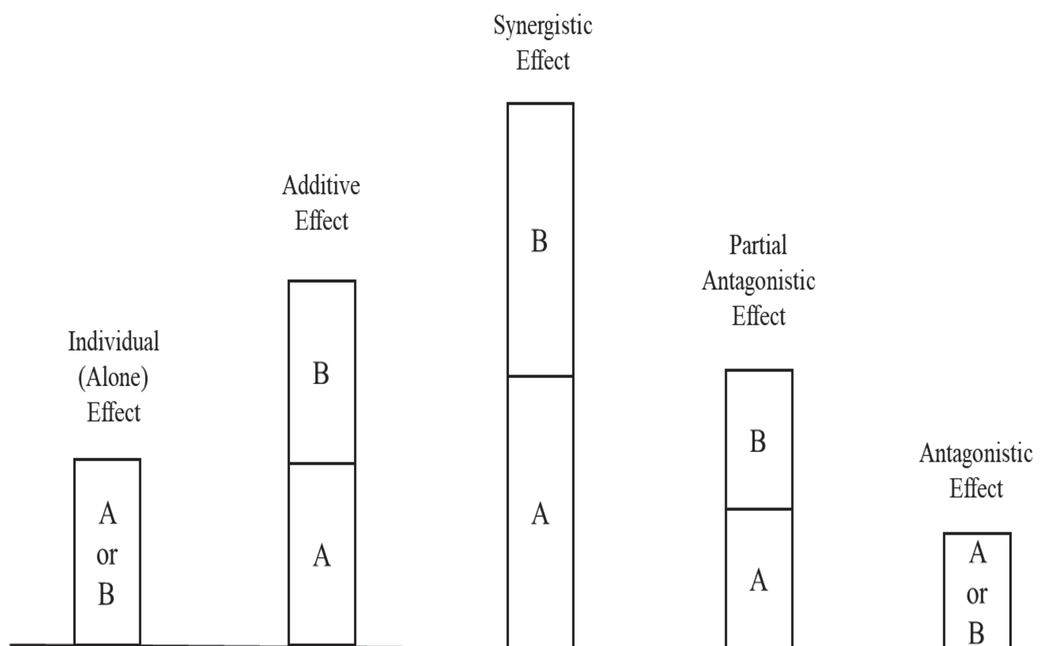


Figure 2.3. Types of hurdle effects

2.4. Fruit Juice Powder

Dehydrated or powdered fruit juice can be defined as "the product obtained from one or several kinds of fruit juice by physically removal of almost all water content" (Alimentarius, 2005; US, 2009). The resulting product is in the powder form and requires the addition of water before use as the fruit juice.

Although fruits and fruit juices are the essential source of the vitamins, minerals and bioactive compounds, their availability are limited due to the seasonal cultivation and harvesting. Fruits and fruit juice are very perishable and sensitive to microbiological, chemical and enzymatic spoilage. However, food manufacturers are focused to produce several forms of the fruit products by using various preservation methods in order to increase their shelf life. Besides, fruit products which have long shelf life can be available for consumers throughout the whole year. These preservation methods must also protect the nutritional quality of the fruit (Cano-Chauca, Stringheta, Ramos, & Cal-Vidal, 2005; Saikia et al., 2014). The fruit juice powders are produced to extend the shelf life of fruit juice, to obtain more stable and easily handled product, and to reduce the size of the packages of products, without affecting the high nutritive contents (Mishra et al., 2014; Roustapour, Hosseinalipour, & Ghobadian 2006). The powders can be used as ingredients, i.e. colouring and flavouring agent, in the food and pharmaceutical industry (Fazaeli et al., 2012; Goula & Adamopoulos, 2010; Shrestha et al., 2007). Ceballos, Giraldo and Orrego (2012) stated that the fruit juice powder is produced mainly to obtain the product that can be easily reconstituted in water. In literature, critical moisture content and water activity of a stable powder were reported to be <4-5% and lower than <0.2-0.25, respectively (Koc, Yilmazer, Balkır, & Ertekin, 2010; Kumar & Mishra, 2004).

The main quality criteria of the dried products are the production of powders that should be low moisture content, low degree of aggregation and high capability of rehydration (Verma & Singh, 2015). The drying of fruit juices are not a simple process due to including of low molecular weight sugars such as glucose, fructose and sucrose and organic acids that have low glass transition temperature (Tg) in their composition. Thus, the sticky and easy caking powders are produced. It can be also encountered the problems such as high hygroscopicity, low melting point and high solubility in these powders (Goula & Adamopoulos, 2010). The loss of product can be also formed due to the stickiness of the product and affect the product yield that is an important parameter in

drying process (Maa, Nguyen, Sit, & Hsu, 1998). All these difficulties are tried to solve in the food industry and drying process became an appropriate for the fruit juices. The stickiness problem can be solved by increasing Tg of the product with using some high molecular weight drying agents such as maltodextrin, starch, gum arabic and soy protein (Jaya, 2002; Caparino et al., 2012; Mishra et al., 2014). Among these drying agents, maltodextrin with several DE content is the most commonly used one because of its lower cost and high efficiency (Rodriguez-Hernandez et al., 2005).

2.4.1. Drying Methods Used for Fruit Juice Powder

Fruits have a high content of water in its composition. Thus, it is prone to spoilage by microorganisms and more sensitive to chemical and enzymatic quality losses (Cano-Chauca et al., 2005). Drying process is one of the most effective, inexpensive and common preservation methods that applied to increase the shelf life of foods (Ratti, 2001; Mishra et al., 2014). Today, dried fruit products gain a place in the market as ready to eat foods that have long term durability (Simha et al., 2012; Singh, Mandhyan, Pandey, & Singh, 2013). However, the objective of the drying of the fruit juice is not only to obtain a stable and easy to use product, but also to produce a fruit juice that has similar quality characteristics with the freshly squeezed ones when it is reconstituted (Mani et al., 2002). Dried fruit juice powders have a lot of benefits and economic potential comparing to liquid form of fruit juices. Decreasing volume and weightiness, lower package size, ease of transportation and longer shelf life are the most common ones (Goula & Adamopoulos, 2010). In addition, physical properties of the fruit juice powders provide a stable, natural and easy to be used as additive, i.e. flavour promoter and colouring agent, in several foods and pharmacologic products (Fazaeli et al., 2012; Shrestha et al., 2007).

The main methods for drying of fruit juices are spray drying, drum drying, vacuum (or vacuum drum) drying, foam-mat drying, fluidized-bed drying, freeze drying and refractance-window drying. The operating principles, advantages and disadvantages of these methods are summarized in Table 2.10.

Spray drying is one of the most common and simple drying methods in the food industry (Verma & Singh, 2015). In spray drying process, liquid and pumpable feeding material is converted to a dried product by using a simple operation. Liquid sample is pulverized by spraying into a chamber by means of a rotating impeller and spraying

nozzle in the dryer. Liquid spraying as droplets is subjected to flow of dryer material that is generally hot air (at 110-210 °C for inlet air). As a result of the heat and mass transport at a very high temperature causing rapid drying, the feeding material has to be low temperature to avoid quality loss in the process. Drying time of droplets is very shorter comparing to other drying treatments (Roustapour et al., 2006; Caparino et al., 2012).

Table 2.10. Drying methods used for production of fruit juice powder

Drying Method	Principle	Advantages	Disadvantages
Spray drying	Atomization of feding liquid and contact of drops with hot air	Low operating cost, large-scale production, and reduced drying time	,Thermal degradation, and formation of agglomerations during drying
Drum drying	Hot roll rotating in the fluid or feed from different rollers	Reduce heat exposure Puffing of the juice on drum	Undesirable cooked aroma Puffing of the juice on drum (vacuum-drum drying)
Vacuum drying	Low temperature drying under vacuum conditions Concentration before drying	Enhance quality by decreasing drying time	Puffing by appyliying rapidly vacuum
Foam-mat drying	Feeding liquid is transformed into foam, which enters into contact with hot air	Reduced drying time, instantaneous rehydration, and low drying temperatures	Non-enzymatic darkening, loss of aromatic components, and produce large volume of foam
Fluidized-bed drying	Feeds liquid disperses on the surface of inert particles	Smaller volume and floor than spray drying Lower investment and operating costs	high gas velocity is needed to fluidize inert gas, thus, power consumption and desing problems
Freeze drying	Freezing water removal from material by sublimation	Excellent nutritional quality, texture, flavor, and color, Low temperature process	High operating cost, long drying time, and high vacuum level
Refractance-window drying (Novel)	In direct contacting of thin spread liquid on a conveyer with hot water	Good nutritional, sensorial and health promoting quality Less energy requirement	Novel drying technique (under investigation)

Drum drying is the other simple process used for powder production in the industry. It is cheaper and more suitable than spray drying for small-scale production due to requiring less space. In this method, one or more rotating hot cylindrical roll or drum are placed within the feeding liquid. The liquid is dried as a thin film by rotating throughout the drums heated with steam or hot water. Finally, powders are collected in

the form of thin layer of the dried liquid (Barbosa-Canovas, Ortega-Rivas, Juliano, & Yan, 2005a). Vacuum drying is another technique applied to fruit juices to reduce the processing temperature (usually at 65-75 °C) and enhance the quality of the powder products. Juice can be concentrated before vacuum drying, and puff-dried powder is obtained by the effect of rapid vacuum pressure (Bates et al., 2001; Jaya & Das, 2004). The powder product obtained after foam-mat drying is similar to vacuum drying. In the foam-mat drying, feeding liquid is converted to puffed powder or foam by adding foaming agent and exposing to hot air. Vacuum and foam-mat drying are preferable methods for fruit juice processing due to obtaining high reconstitutable powders in water (Bates et al., 2001; Franco, Perussello, Ellendersen, & Masson, 2016; Kadam, Wilson, Kaur, & Manisha, 2012). In fluidized-bed drying, feeding liquid is spread onto a bed including inert particles. Then, a bridge occurs after wetting and come together of the inert particles. Powders are obtained from the solid form of this bridge due to heating effect. This technology has a limited use due to requiring high gas velocity and power to fluidize inert particles (Cabral, Telis-Romero, Telis, Gabas, & Finzer, 2007; Jinapong, Suphantharika, & Jamnong, 2008).

Among all of the drying methods, freeze drying is the most suitable method for preservation of the quality of a powder product due to processing at low temperatures. The method is based on the removal of the ice form of water in the juice by sublimation. However, this method is very expensive compared to other methods. The principles, advantages, limitations and applications of the freeze drying method are explained in the following section (2.4.1.1.) in more detail.

Recently, refractance-window drying is being developed as a novel drying method. This method is based on the drying of a thin liquid spread on the conveyor belt by indirectly heating with a hot water (95-97 °C) and short time. Thus, powders have good nutritional, bioactive and sensorial quality due to indirectly heating (Baegbali, Niakousari, & Farahnaky, 2016; Caparino et al., 2012). The refractance-window drying method may be an alternative to freeze-drying because it is more economical.

2.4.1.1. Freeze Drying

Different drying techniques can be used for removal of the water from a food material. However, the shrinkage that takes place during classical dehydration process

prevents rehydration and produces products with lower apparent density and higher porosity. Freeze drying method is the best water removing technique to provide high quality dried product (Shishehgarha et al., 2002). The basic principle of the freeze drying is the removal of ice from a material through the process of sublimation and the removal of bound water molecules through the process of desorption and leaving the solutes in their almost anhydrous form (Franks, 1998). The basic freeze dryer consists of a drying chamber, vacuum pump, condenser, compressor and controller with the addition of auxiliaries (Figure 2.4).

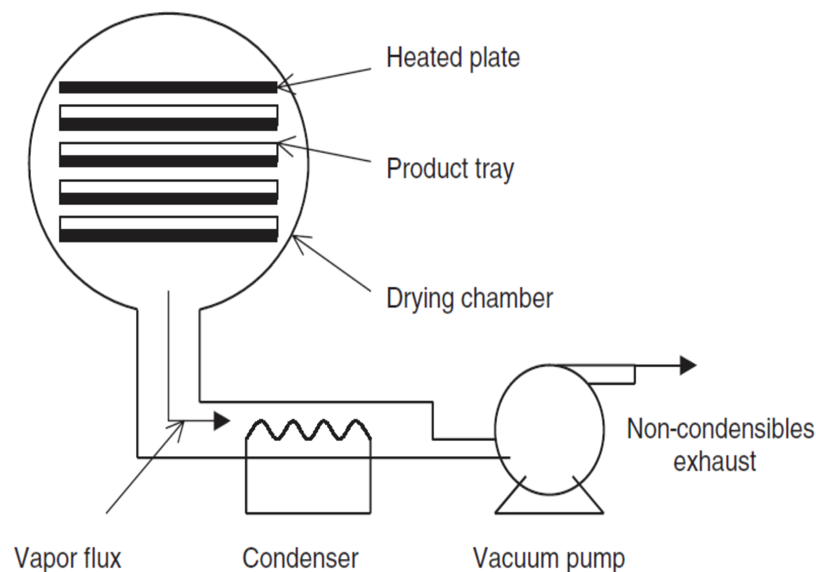


Figure 2.4. Basic configuration of freeze dryer
(Source: Liapis & Marchello, 1984)

In the freeze drying process, the drying liquid or aqueous material is put into glass vials of the dryer. These vials are placed into the cooled shelves of the dryer. Product material is frozen uniformly in this part. After freezing step, pressure in the drying chamber is set up the lower level of the ice vapour pressure at local temperature to start the freeze drying of the product. Removal of moisture in the product is mainly achieved by sublimation during freeze drying step. In the freeze dryer, shelf temperature during drying should be as high as required for efficient sublimation process but also not as low as to avoid melting of the frozen product at the bottom of the vials (room temperature is ideal) (Wang, Chen, & Chen, 2012).

There are three main steps in the freeze drying process. These are freezing (solidification), primary drying (ice sublimation) and secondary drying (desorption of

unfrozen sorption) steps. In the freezing step, water phase in the liquid product became to the form of ice crystals and more water converted to ice form by continuing freezing. Thus, the liquid part of the material becames more concentrated and the viscosity of the liquid product increases in this step. 65-90% of water content of the initial product can be frozen during freezing step. The small amount of water part in the liquid material that is known as bound water is not frozen in the initial freezing step (Abdelwahed, Degobert, Stainmesse, & Fessi, 2006; Wang et al., 2012). After freezing, ice in the frozen product is removed via sublimation under vacuum in the primary freezing step. Two parameters as temperature and pressure can be controlled in this step. The rate of ice sublimation from the product depends on the vapour pressure of the product and the vapour pressure of the ice collector in the drier equipment since the ice molecules are moved from the high pressure (in product) to low pressure (in collector) area (Nireesha et al., 2013). However, there will be some water bounded in the product. In the final step of freeze drying (secondary freezing), about 2% w/w of remaining water in the product is removed by desorption. This desorption occurs by decrease the water content in the product without any change of volume and the final product becomes more stable (Przic, Ruzic, & Petrovic 2004).

Freeze drying is a process that has many advantages comparing to other drying techniques. The most important ones are better stability of the product, easy handling (shipping and storage), and excellent product quality due to the low temperature process and little contamination due to aseptic process. However, high cost as well as time and energy consuming rates of the freeze drying process limit its application (Erbay & Kucukoner, 2008; Kasper & Friess, 2011; Ratti, 2001; Tang & Pikal, 2004). It was stated that freeze drying is four or five times more expensive when compared to spray drying method (Michalska, Wojdylo, Lysiak, & Figiel, 2017).

Freeze drying process is widely used for pharmacological, biological materials, nanomaterials as well as foods. In food industry, freeze drying is the widely applied to produce instant coffee powder. Other foods can also be freeze dried to be used as additives (berries for breakfast cereals) or ready to eat products (eatable ice cream without reconstitution, less weight backpacking foods such as freeze-dried beef, pasta, meat, fish) and organoleptical foods (some aromatic herbs) (Hammami & Rene, 1997; Przic et al., 2004; Ratti, 2001). Seasonal of fruits and vegetables are often dried to increase their availability all year around (Marques et al., 2006; Ratti, 2001).

2.4.2. The Effect of Different Drying Methods on the Quality Properties of Fruit Juice Powder

Fruit juice powder can be produced by using several drying methods (Table 2.10) (Bates et al., 2001; Michalska et al., 2017). Final powder product obtained by these methods using different operational conditions has different quality properties. Spray drying is the most widely used one in the fruit juice industry. However, this method is applied in a short time at high air temperature, thus, some quality losses occurs in the powder product (Roustapour et al., 2006). In drum drying, cooked flavour occur in the product due to being exposed to steam at high temperature (120-170 °C) within the drum (Caparino et al., 2012). Vacuum drying is the other high temperature drying method but operating temperature is relatively reduced with vacuum. Better retention of quality is provided in the powder product with a reduced thermal degradation (Michalska et al., 2017). Freeze drying is the best method used to obtain high quality powders among all drying methods. Freeze dried juice powders are considered to have excellent quality properties because they are obtained at low temperature process conditions (Ratti, 2001). Thus, the nutrients and bioactive properties in the juice composition are well-preserved in freeze dried form (Franceschinis et al., 2014). Freeze dried powders have also good rehydration capacity, colour, flavour, aroma, and taste characteristics. They are expected to be more nutritive but more expensive because of the high production cost (Pavan, Schmidt, & Feng, 2012).

There are too many studies related to the effects of several drying methods on physical, nutritional, sensorial and bioactive quality parameters of fruit and fruit juice powder products. Many of them are related to spray drying method. The properties of the dried products varies in terms of selected drying conditions. Generally, drying processing at lower temperature and in a shorter time better preserves the quality of the powders. For example, Kha, Nguyen, and Roach (2010) studied spray drying of gac aril fruit juice powder at different inlet air temperatures (120, 140, 160, 180, 200 °C). They indicated that carotenoid content and antioxidant activity of the juice were better preserved after spray drying at 120 °C inlet air temperature and 56 ±2 m^3/h air flow conditions. Antioxidant activity and anthocyanin content of the powders depend on the process conditions; for example, spray drying process at low temperature had little effect on the antioxidant and anthocyanin content of gac fruit aril powder and pomegranate juice

powder (Kha et al., 2010; Vardin & Yaşar, 2012). Vardin and Yasar (2012) studied spray drying of pomegranate juice using inlet temperatures ranging between 80-200 °C. They obtained high quality powders after processing at temperature at 120-145 °C. Kadam et al. (2012) produced tomato juice powder by using foam mat drying at different temperatures (65, 75, 85 °C) and found that the nutritive value of the powder was not changed significantly by this technique. Singh et al. (2013) observed that ascorbic acid content of the juice could be also conserved by applying spray drying at low temperature. Ascorbic acid was reduced from 54.6% to 24.5% in spray-dried ber juice by increasing inlet air temperature from 170 to 210°C (Singh et al., 2013). In another study, nutrients and colour property of the water melon juice were well-preserved in the powder by using spray drying temperature less than 165 °C (Quek, Chok, & Swedlund, 2007). Baeghbali et al. (2016) stated that refractance-window drying performed by circulating hot water at 91 °C and flow rate of 2.5 m^3/h can preserve bioactive and color properties of pomegranate juice powder. Caparino et al. (2012) found that drum drying (at 152 °C for 54 s) in mango puree resulted in the dark powder color due to application of drying process for a long time at high temperature.

It was revealed that the flavour components of the powders dried at high processing temperature are different than fresh fruits due to the degradation of some volatile components during dehydration (Marques et al., 2006). Carrier concentration is also an important processing parameters in the quality of fruit juice powder. It was indicated that the nutritive value and colour of the fruit juice powders can be protected using some drying agents such as maltodextrin, gelatin, starch, and gum arabic (Lee, Wu, & Siow, 2013). There are several studies in the literature investigating the effect of carrier concentration on the quality of the fruit juice powders (Abadio, Domingues, Borges, & Oliveira, 2004; Cano-Chauca et al., 2005; Caparino et al., 2012; Lee et al., 2013; Oberoi & Sogi, 2015; Vardin & Yasar, 2012).

Freeze drying is found to be the most appropriate method in order to protect antioxidant content, vitamins and other nutritional quality of fresh fruits and vegetables (Pavan et al., 2012). The method is suggested if the ascorbic acid content is selected as important quality criteria for the fruit juices (Ceballos et al., 2012). Table 2.11 summarizes the studies related to the effect of freeze drying on the quality characteristics of various types of fruit juices. Freeze drying method was also compared to various types of drying methods in Table 2.11.

Table 2.11. The summary of the freeze drying studies of fruit juice

Juice	Freeze drying conditions	Findings on the powder quality	Reference
Cactus pear juice	Juice/Maltodextrin (5:1) 72h drying	No adverse effect in color, NEBI and HMF content 10% loss of vitamin C	Moßhammer et al. (2006)
Blackberry juice	Juice/Maltodextrin (80:20) 48h drying (-84 °C/0.04mbar)	Best retention of bioactive compounds and antiradical activity Lower physical stability than spray dried powder	Franceschinis et al. (2014)
Lime Rangpur juice	No agent From 1 to 10 h (-61 °C/3 Pa)	Preserved physicochemical and sensorial properties	Acevedo et al. (2014)
Pomegranate juice concentrate	Gum arabic (35%) 24h drying (-40 °C/3 Pa)	Preserving of total anthocyanins content, anthocyanin color and antioxidant activity in the reconstituted pomegranate juice The least color change than refractance and spray drying	Baeghbali et al. (2016)
Black current juice	Maltodextrin, Gum arabic Tapioca starch (adding agent from 6 to 20 °brix) ?h drying (-53 °C/0.02 mbar)	Better retaining of anthocyanin and antioxidant activity when using maltodextrin The better anthocyanin and color properties than spray dried powder	Murali et al. (2014)
Blueberry juice	Maltodextrin β-cyclodextrin (%15 w/w) (-50 °C/0.042 mbar, then, -50 °C/0.05 mbar)	1.5 fold better retention of anthocynanins in freeze dried powder than in spray dried one	Wilkowska et al. 2016
Frozen mango puree	No agent 31h drying (-60 °C/20 Pa)	Color best preserved in freeze drying than drum and spray drying Porosity in freeze-dried particles	Caparino et al. (2013)
Water melon juice	Maltodextrin (3, 5, 7, 10%) ?h drying	Retaining more pigments in freeze-dried powder than spray drying Limited shelf life due to higher water activity than spray dried powders	Oberoi and Sogi (2015)
Açai juice	No agent	Good stability during storage (high Tg) Lower moisture content and a_w than refractance-window drying	Pavan et al. (2012)

2.5. Concentrated Fruit Juice

Codex Alimentarius Commission defined the concentrated fruit juice as "the product obtained from the fruit juice where the water content of the juice has been physically removed in an amount sufficient to increase the Brix level to a value at least 50% greater than the Brix value established for reconstituted juice from the same fruit (Alimentarius, 2005). The same definition of the concentrated fruit juice was also published in a Regulation of Turkish Food Codex (communique number of 29080) (Turkish Food Codex, 2014).

Fruit juice from concentrate is also described as the juice which has been concentrated (by evaporation under reduced pressure) and returned to its original state by the addition of water. Flavour lost during the process has to be restored to produce an end product which displays organoleptic and analytical characteristics at least equivalent to those of an average type of fruit juice obtained from fruit or fruits of the same kind. Pulp and cells recovered during the process of producing the fruit juice in question or fruit juice of the same kind may also be restored to the juice (Turkish Food Codex, 2014).

2.5.1. Evaporation Methods Used for Fruit Juice Concentrates

The concentration of fruit juice provides a lot of advantages such as reduced weight and volume, and packaging cost, easy transportation, handling, and storage, and more stable product due to low water activity (Cassano & Drioli, 2007).

Concentration methods for the fruit juices are listed generally as open-pan boiling method, thermal vacuum evaporation, reverse osmosis, osmotic evaporation, membrane technology and electrodialysis (Bates et al., 2001; Yaakob, 2012). The general principles, advantages and limitations of these evaporation methods used for fruit juice are summarized in Table 2.12.

Among these evaporation methods (Table 2.12), open-pan boiling has been used as a traditional method for a long time. Water in the juice is removed from by boiling at the atmospheric pressure within an open pan. This method is a time and energy consuming process, and undesirable heat damage or severe quality loss occurs in the concentrated products.

Table 2.12. Evaporation methods used for production of concentrated fruit juice (Source: Bates et al., 2001; Yaakob, 2012)

Evaporation Method	Principle	Advantages	Limitations
Open-pan boiling	Boiling of water at the atmospheric pressure	Economical and simple method	Long time process High energy required Low quality product
Vacuum evaporation	Reduce boiling point of water under vacuum pressure	Energy efficient Reduce heat damage Good quality product	Costly equipment Loss of aromatic compounds and vitamin
Reverse osmosis	Remove of water (solvent) under osmotic pressure Solution of fruit juice through out to membrane.	Low temperature process High quality product Better preserved aroma	Use high operating pressures in order to overcome the osmotic pressure of the juice ranging from 10 to 200 bars Slow Process Limited concentration (up to 40 °Brix)
Osmotic evaporation	Removing of water by hydrophoic membrane Use of a hydrophobic microporous membrane to separate two liquid phase that differer greatly in terms of solute concentration	Suitable for processing of heat-sensitive aqueous solutions such as fruit juices	High energy consumption For must rectification, they require an additional operation of ion-exchange, which leads to severe ecological problems due to resin regeneration and disposal Juice must be clarified before Efficient concentration (up to 65 °Brix)
Membrane technology	Cross flow microfiltration (CMF) has been applied successfully to some highly thermo sensitive juices, resulting in microbiologically stabilized clarified juice that preserves the major part of the fruit"s original aroma	Suitable for thermosensitive juices including special aroma	Same as osmotic evaporation because it use of a hydrophobic microporous membrane as their filtrate.
Freeze concentration (cryoconcentration)	The separation of soluble solids from a liquid phase by means of freezing the water content of the liquid	Operating at low temperature Excellent product quality Minimizes losses of volatiles and thermolabile components	Expensive equipment High-cost and slow process Clear solids attainable Limited concentration (up to 40 °Brix)
Electrodialysis (pervaporation)	The separation or concentration of ions in solution	High quality product	Expensive equipment High-cost and slow process Clear solids only

The most common concentration process is the vacuum evaporation which the water in the fruit juices is removed by using high temperature heat treatment (Barbe et al, 1998; Yaakob, 2012). However, the product obtained from this evaporation can have cooked or off-flavours when it was applied at high temperatures. This method is explained in detail in the following section (section 2.5.1.1). Besides, nonthermal concentration techniques are developed to avoid the degradation of the ingredients in the juice composition to provide better quality. Freeze concentration (cryoconcentration) and membrane techniques are among these methods (Cassano & Drioli, 2007; Celere & Gostoli, 2004). In freeze concentration, water is removed as in the ice form and the process is carried out at low temperature. Although the flavour compounds are protected excellently in freeze concentration, the use of the technique is limited by the reason of the high operation costs and low water removal efficiency (Yaakob, 2012). Membrane concentration processes such as reverse osmosis, osmotic evaporation, membrane evaporation and electrodialysis are the other alternative concentration techniques to overcome adverse effects of the thermal evaporation. These methods did not include phase transition of water. But high energy consumption and high cost are their disadvantages (Cassano & Drioli, 2007; Yaakob, 2012). In osmosis theory, water in the low solid concentration solution moves to high solute concentration solution by using a semipermeable membrane. Therefore, osmotic pressure occurs. In reverse osmosis evaporation, high hydraulic pressure must be applied to overcome osmotic pressure in the juice. Thus, water is transported from high solid to low solid concentration. Heat-sensitive aroma components are better protected at low processing temperature of reverse osmosis (Yaakob, 2012; Girard & Fukumato, 2000). In osmotic evaporation (or osmotic distillation), two liquid phases having different solute concentration are differentiated each other by using a hydrophobic microporous membrane. Hydrophobic membrane does not allow the penetration between pores and aqueous solutions, and air gaps occur. Partial pressure is generated in the vapour phase based on the differences in water activity values between two sides of membrane. Therefore, vapour in the liquids moves across the pores from the high to the low pressure phase. Fruit juice can be concentrated up to 65 °Brix by osmotic evaporation method (Vaillant et al. 2001). Membrane evaporation (or membrane distillation) has similar principle as osmotic evaporation, i.e. hydrophobic membrane is used. The only difference between the osmotic and membrane evaporation methods is the drving force occurred. In membrane evaporation, vapour pressure gradient is generated by temperature difference across the membrane, while in osmotic evaporation, this

pressure is depended on concentration difference (Bahmanyar, Asghari, & Khoobi, 2012; Bahceci, Akıllıoglu, & Gokmen, 2015). Electrodialysis (pervaporation) is another membrane technique used in concentration of fruit juice. This method is different than the other pressure-driven membrane technologies. The positive and negative ionic charges of the solution is transferred across the ion-exchange membranes (Girard & Fukumoto, 2000; Lopez-Leiva, 1988).

2.5.1.1. Vacuum Evaporation

Thermal vacuum evaporation is the most widely used, most efficient and economical method for the concentration of fruit juice employed by the fruit juice industry (Bates et al., 2001; Rizvi, 2010). This method is applied to overcome thermal damage occurred in the juice concentrates obtained by open-pan boiling method. Vacuum evaporated juice has better preserved flavour, nutrients and color due to lower temperature application (Fellows, 2017).

Vacuum evaporation method is based on the lowering boiling point of water under vacuum pressure conditions. Water boils when the saturated vapor pressure of water is equal to the external pressure on the water surface (100 °C at the sea level). At the conditions of lower pressure than atmospheric environment, vacuum occurs and water boils at lower temperatures (Fellows, 2017). In the vacuum evaporation, water in the juice can be partially removed in the form of vapour at a lower processing temperature (<100 ° C), while soluble solid content such as vitamins, minerals and sugars are left in the juice composition. After evaporation, more concentrated product with high soluble solid content is produced (Assawarachan & Noomhorm, 2010). Besides this method can be also applied for pre-concentration, volume reduction, water/solvent recovery and cyristallization purposes (Eagri, 2018).

In the vacuum evaporation system, the evaporation rate of the juice is affected by processing conditions, types of evaporator and juice properties (Assawarachan & Noomhorm, 2010). Generally, the evaporation of juice is applied between 60-65 °C by using falling film and rising film type evaporators in the industry (Barrett et al., 2004). Typical evaporators has several steps including heating of the juice and cooling it immediately (Lin, Rouseff, Barros, & Naim, 2002). Basic configuration of a vacuum evaporator is demonstrated in Figure 2.5. This equipment is composed of a steam source,

a heat exchanger, a feed distributor, vacuum creator, vacuum separator and condenser. Food product is fed to the system and steam is transferred to the food by a heat exchanger. Water is removed in the vapour form from the separator under vacuum pressure and concentrated product is collected from the evaporator. In some cases, volatile compounds can be recovered in order to be added to the concentrated juice after evaporation (if the boling point of aroma compounds is lower than boiling point of water) (Fellows, 2017). The efficiency of the vacuum evaporation can be improved by using multiple effect evaporator systems performed at maximum 50 °C operating temperature. In these systems, steam of the first effect is used as a heating medium in the second effect of the system, and so on (Barrett et al., 2004).

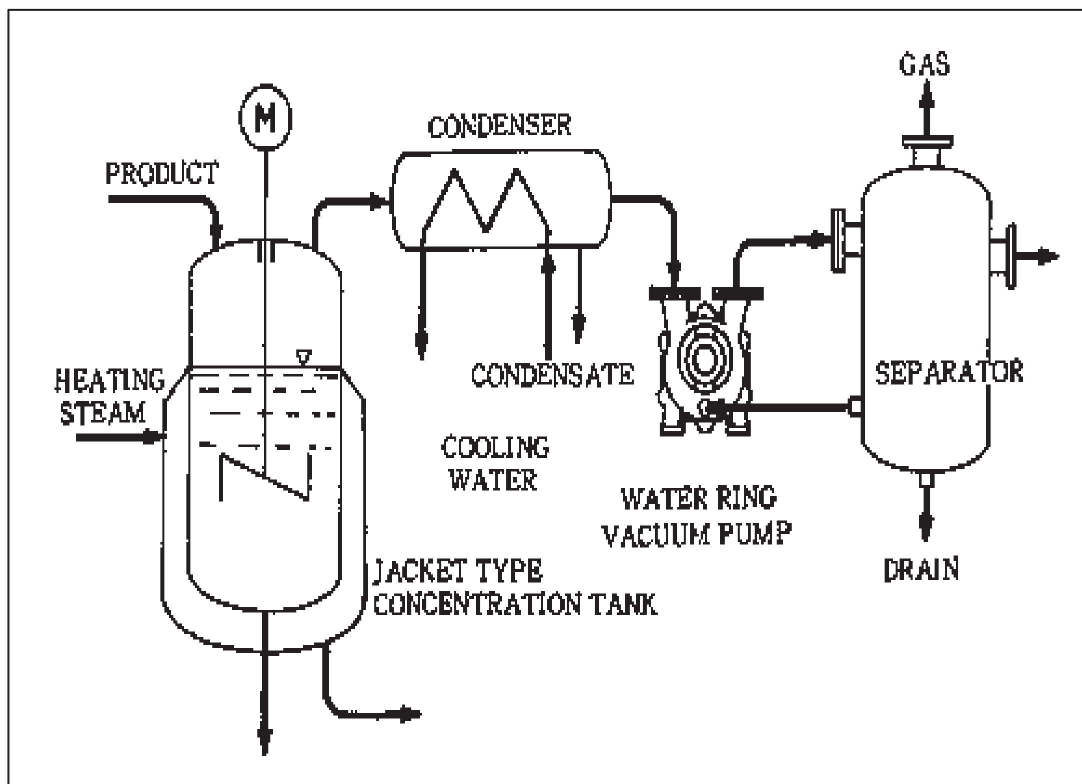


Figure 2.5. Basic configuration of vacuum evaporation equipment

2.5.2. Effect of Evaporation Methods on the Quality Properties of Concentrated Fruit Juice

Fruit juice concentration is one of the main unit operation that enable fruit products to be processed in the industry. Final form of the concentrated fruit juice product

can be used as an ingredient in ice-creams, fruit syrups, jellies and fruit juice beverages. Therefore, the characteristics of the final product such as color, flavor, aroma, appearance and mouthfeel are very important (Cassano, Jioa, & Drioli, 2004; Maskan, 2006). These properties may vary depending on the processing conditions of evaporation methods. In the literature, there are several evaporation methods applied at different conditions to fruit juice. The most common one is thermal vacuum evaporation method. Table 2.13 summarizes the studies related to the vacuum evaporation method in the literature. New techniques are also developed and compared to vacuum evaporation method by considering the quality parameters of the concentrated fruit juice products (Bozkir & Baysal, 2017; Cassano & Drioli, 2007; Cisse et al., 2011; Dincer, Tontul, & Topuz, 2016; Fazaeli, Hojjatpanah, & Emam-Djomeh, 2013b; Maskan, 2006; Najafabadi, Sahari, Barzegar, & Esfahani, 2017; Yousefi, Emam-Djomeh, Mousavi, & Askari, 2012).

For example, Cassano and Drioli (2007) compared the osmotic distillation and vacuum evaporation methods for concentrating clarified kiwi fruit juice. They reported that ascorbic acid content and antioxidant activity of the concentrate (66.6 °Brix) did not change by osmotic evaporation (evaporation flux of 1.3 $kgh^{-1}m^{-2}$, 25 °C). However, they detected 87% and 50% losses for vitamin C and antioxidant activity, respectively, after vacuum evaporation of kiwi juice at 75 °C. Fazaeli et al. (2013b) studied with the effects of microwave, thermal vacuum and atmospheric evaporations on the quality of black mulberry concentrates. They found that degradation of anthocyanins in the thermal vacuum evaporation (7.3 kPa) (k: 0.066 h^{-1}) was two times faster than in the microwave evaporation (300 W, 7.3 kPa) (k: 0.034 h^{-1}). This was due to short evaporation time of microwave method. Besides, the highest rate of degradation were detected in the concentrates obtained by boiling at atmospheric pressure (k: 0.348 h^{-1}). Similarly, Maskan (2006) investigated the effect of microwave (350 W), vacuum (40 °C) and atmospheric evaporation (open-pan boiling) methods on the color properties of pomegranate juice concentrate. They obtained higher color degradation in the products concentrated by vacuum evaporation method. This was attributed to inadequate inactivation of oxidative enzymes at low-temperature vacuum conditions. Cisse et al. (2011) compared the quality properties of apple and grape juice concentrates obtained by osmotic (evaporation flux of 1.17 $kgh^{-1}m^{-2}$, 35 °C) and vacuum evaporation (80 °C/50 kPa) methods. They indicated that physicochemical, biochemical and aromatic properties of the juices were better preserved by osmotic evaporation method.

Table 2.13. The summary of the vacuum evaporation studies of fruit juice

Juice	Vacuum evaporation conditions	Findings on the concentratred quality	Reference
Blackberry juice 8.9 °Brix	60 °C/less than 100 min by rotary vacuum evaporator Final TSS: 65 °Brix	There was less than 5% loss of anthocyanins after concentration Degradation of anthocyanins in the concentrate at 25 °C was much more faster than 5 °C storage	Wang and Xu (2007)
Kiwi fruit juice 9.4 °Brix	75 °C by rotary vacuum evaporator Final TSS: 66.6 °Brix	87% reduction of ascorbic acid 50% reduction of total antioxidant activity	Cassano and Drioli (2007)
Black mulberry juice 16 °Brix	-100 kPa (Atmospheric)/140 min - 38.5 kPa/120 min - 7.3 kPa/95 min by rotary vacuum evaporator Final TSS: 42 °Brix	Anthocyanin degradation and color change due to pigment destruction occurred at the maximum rate at atmosphheric evaporation	Fazaeli et al. (2013b)
Pomegranate juice 17.5 °Brix	-Atmospheric boiling/190 min -40 °C /66 rpm/108 min by rotary vacuum evaporator Final TSS: 60.5 °Brix	Pigment destruction and dark color formation occurred at atmospheric evaporation More color change occurred in vacuum evaporation due to not inactivating oxidative enzymes at low temperature (40 C°)	Maskan (2006)
Pomegranate juice 19.2 °Brix	-93.5 °C/100 kPa (Atmospheric) /140 min - 74 °C/38.5 kPa/127 min - 51 °C/12 kPa/109 min by rotary vacuum evaporator Final TSS: 65 °Brix	Anthocyanin degradation at atmospheric evaporation was at least 7 times faster than vacuum evaporation processes Nonenzymatic browning reactions decreased by vacuum evaporation Antioxidant capasity is better preserved in vacuum evaporation	Yousefi et al. (2012)
Apple juice 11 °Brix Grape juice 15.7 °Brix	80 °C/50 kPa by rotary vacuum evaporator Final TSS: 66 °Brix (grape) 57 °Brix (apple)	Sugar and acidity content decreased by heating due to degradation reactions Only 6% anthocyanin loss Aroma compounds lost Furan and HMF were observed	Cisse et al. (2011)
Apple juice 11 °Brix	82 C/50 kPa by rotary vacuum evaporator (VE) and rising film evaporator (RFE) Final TSS: 66.5 °Brix (grape)	Total color difference was the highest level in RFE The lowest total phenolic content obtained in RFE The highest HMF found in RVE	Bozkir and Baysal (2017)
Jujube juice 25.3 °Brix	- 98 °C/(Atmospheric)/117 min - 40 °C/96 min by rotary vacuum evaporator Final TSS: 65 °Brix	Color degradation of concentrates during storage (4 °C/90 days) Higher anthocyanin loss (25.6%) found in atmospheric evaporation than vacuum evaporation (17.6%) during storage	Najafabadi et al. (2017)
Black mulberry juice 14.5 °Brix	80 °C/25kPa//100 rpm/120 min by rotary vacuum evaporator Final TSS: 65 °Brix	Turbidity increased by polymerization of phenolics Anthocyanin loss was 8.5% HMF not detected after process but it was formed during storage even at 4 °C	Dincer et al. (2016)

Generally, the quality properties of the concentrated fruit juice was better preserved by means of microwave vacuum evaporation method performed within a shorter time than thermal vacuum evaporation, or membrane evaporation methods (osmotic evaporation, reverse osmosis etc.) processed at the low temperatures. However, the open-pan evaporation at atmospheric pressure was resulted in the lowest quality product. The quality of the concentrated product can be improved by a vacuum evaporation method with suitable combinations of vacuum pressures and temperatures.

2.6. Grape and Verjuice

2.6.1. The Production of Grape in the World and Turkey

Figure 2.6 shows the most important countries for grape production with their percentage of production. It is understood that China (19%), Italy (11%) and the United States (9%) are the leaders of grape producers in the world. Turkey can be also considered as a leader country by providing 5% of the grape production in the world. Additionally, Turkey has an important place in the world with a share of 5% among the grape exporting countries (Figure 2.7).

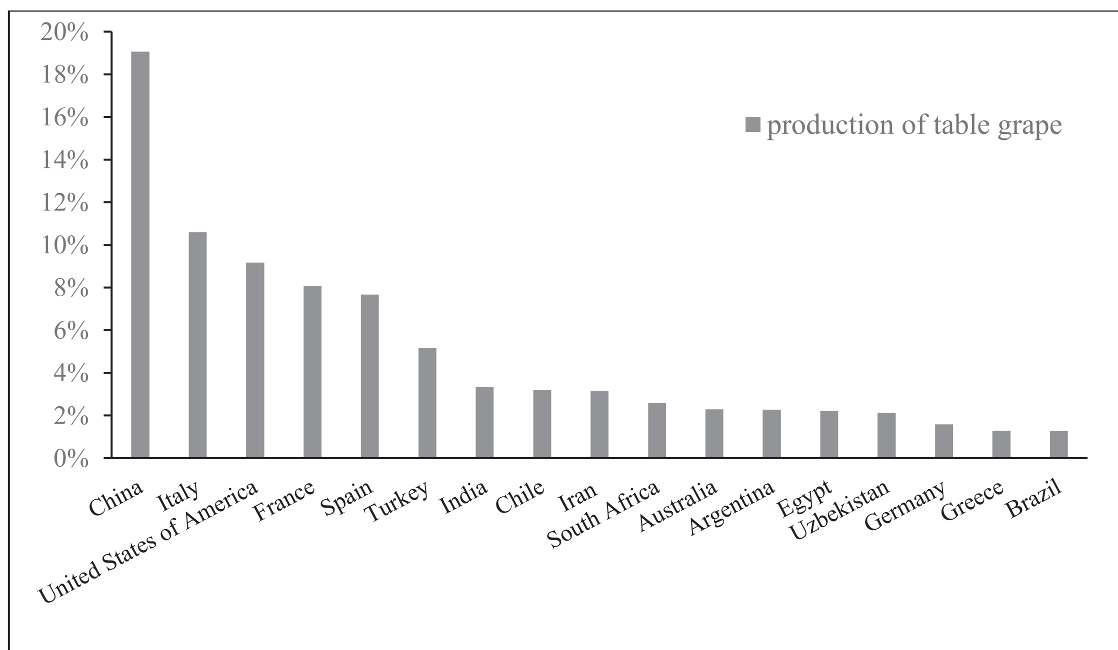


Figure 2.6. Grape production in the world in 2016
(Source: FAOSTAT, 2018)

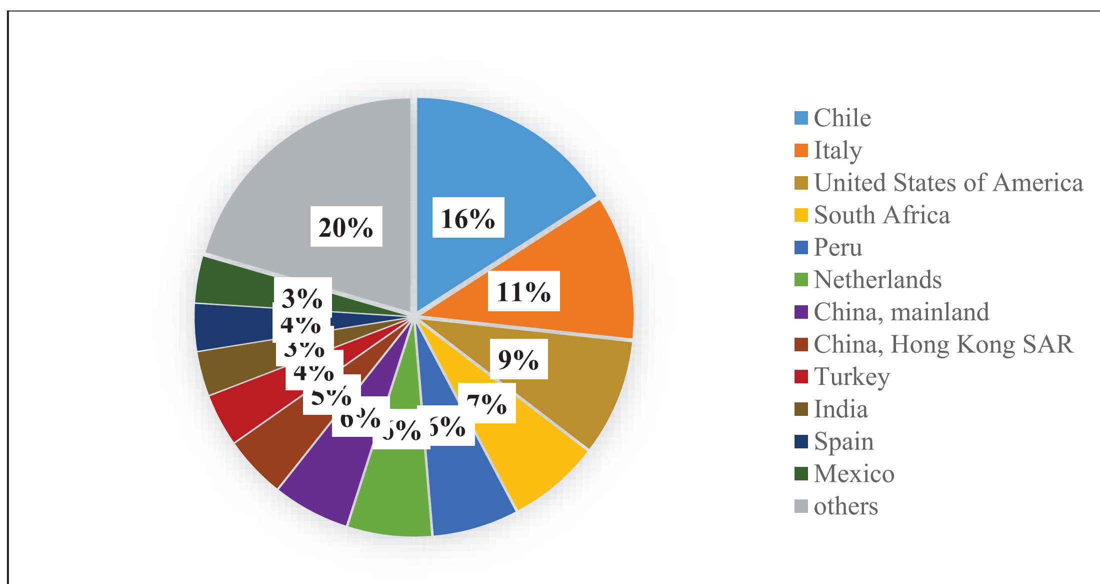


Figure 2.7. Grape exporting countries in the world in 2016
(Source: FAOSTAT, 2018)

Among a large variety of fruits produced in Turkey, grape has the highest production rate (4265-3650 kilotonnes). Therefore, grape and its products (grape juice, concentrate, raisin, wine etc.) are becoming increasingly important in the market of Turkey. The grape production in Turkey from 2000 to 2017 is demonstrated in Table 2.14. According to table 2.14, grape was produced in the high amount than the requirements (>100% self-sufficiency) from 2009 to 2017. It was concluded from these reports that grape production in Turkey is sufficient to meet the requirements for the consumption of the fruit and fruit products such as grape juice, concentrate or other grape-based products. Nevertheless, some amount of grapes are lost during processing or transportation of the fruits. Considering the production in 2017, 2288 kilotonnes (57.5%) grape was used for human consumption. Besides, 8.1 (0.2%), 1231 (30.9%) and 235.2 (5.9%) kilotonnes were used for import, export and industrial usage (Figure 2.8). However, approximately equal amount of grape used for the industrial usage was recorded as the losses (5.5%) in Figure 2.8.

Grape production in Turkey is seperated for the use of stone and seedless table grape, stone and seedless raisin and wine. The percentage of grapes used for these products is given in Figure 2.9. Table and raisin varieties of grapes are also used in the production of alcolic beverages (raki, liquor etc.) and nonalcolic beverages such as verjuice (Hayoglu et al., 2009).

Table 2.14. The grape market in Turkey
(Source: Turkstat, 2018a)

Marketing year	Production (kilotonnes)	Imports (kilotonnes)	Exports (kilotonnes)	Human consumption (kilotonnes)	Human consumption per person (kg)	Industrial use (kilotonnes)	Losses (kilotonnes)	Degree of Self-sufficiency (%)
2016/'17	4,000	8.1	1,231	2,288	28.7	235.2	219.4	141.6
2015/'16	3,650	14.3	1,003	2,072	26.3	241.3	201.1	141.0
2014/'15	4,175	15.7	1,296	2,312	29.8	246.6	222.5	145.8
2013/'14	4,011	14.6	971	2,507	32.7	246.6	239.5	130.1
2012/'13	4,234	24.0	1,187	2,389	31.6	261.0	230.4	142.8
2011/'12	4,296	15.5	1,123	2,598	34.8	259.3	248.4	134.3
2010/'11	4,255	8.9	1,093	2,538	34.4	273.3	244.4	135.2
2009/'10	4,265	14.5	1,084	2,548	35.1	254.2	243.7	136.0
2008/'09	3,918	10.3	1,241	2,099	29.3	259.4	205.1	148.4
2007/'08	3,613	22.8	1,065	2,080	29.5	256.1	203.2	138.1
2006/'07	4,000	22.6	1,203	2,228	...	317.6	221.3	140.4
2005/'06	3,850	19.8	1,008	2,275	...	304.4	224.3	133.3
2004/'05	3,500	12.6	1,101	1,843	26.1	250.2	182.0	149.4
2003/'04	3,600	7.1	856	2,137	30.6	290.2	211.1	132.5
2002/'03	3,500	6.9	951	2,013	29.2	273.4	198.8	136.7
2001/'02	3,250	4.7	940	1,825	26.9	247.8	180.2	140.1
2000/'01	3,600	12.6	917	2,068	30.9	280.8	204.2	136.9

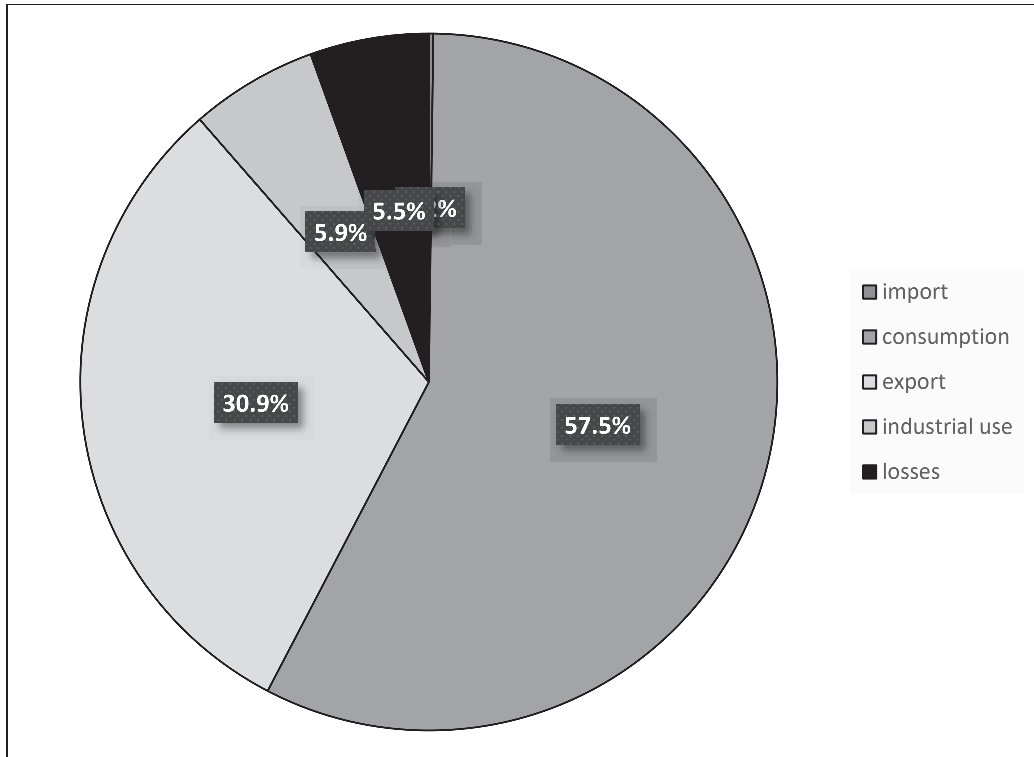


Figure 2.8. Production of grape in Turkey in 2017
(Source: Turkstat, 2018a)

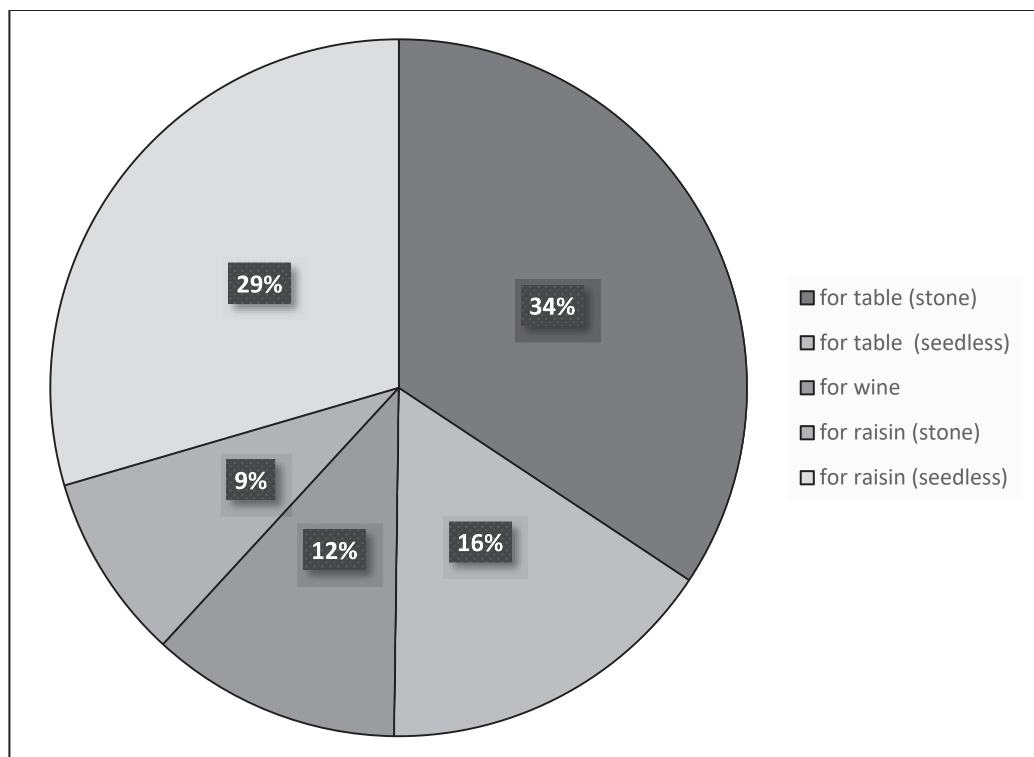


Figure 2.9. Use of grape in Turkey in 2017
(Source: Turkstat, 2018a)

2.6.2. Raw Material of Verjuice: Unripe Grape (Koruk)

Koruk, which is also known as “sour grape” or “unripe grape”, can be defined as the grapes that are harvested before ripening. The Koruk is commonly grown and used in the Mediterranean and Southeastern Anatolia region (Hayoglu et al., 2009). However, Australia, New Zealand, South Africa, USA and Spain are the pioneer countries in the world for unripe grape cultivation and usage (Beer, 2003).

Koruk has sour taste because it has high acidity. The amount of sugars such as glucose and fructose is also lower in the Koruk compare to the mature grapes. Thus, it is not suitable for consumption as table grapes (Balaswamy, Rao, Nagender, & Satyanarayana, 2011; Soyer et al., 2003). *Vitis vinifera* species of grapes, i.e. Yediveren (generally), Kabarcık, Thompson seedless, are used in the preparation of sour grape juice named as “verjuice”. The harvesting time of Koruk is determined based on its “maturation index” (total soluble solid content [bx°] / acidity [%] ratio) (Palomo, Diaz-Maroto, Vinas, Soriano-Perez, & Perez-Coello, 2007). In Turkey, harvesting season of Koruk is usually between June and July.

Koruk is commonly used in the production of verjuice and various drinks, and as an ingredient to enhance the flavour of traditional meals, salads and appetizers (Hayoglu et al., 2009; Hildebrandt & Matchuk, 2002; Karapinar & Sengun, 2007). Sour grape is also used as a raw material in the production of long-shelf life products such as raisins, jam, spread, sweet chutney and canned grapes (Rao, Balaswamy, Velu, Jyothirmayi, & Satyanarayana, 2009).

2.6.3. Verjuice

Verjuice is defined as an unfermented grape juice which is obtained by squeezing directly of unripe table grapes. Unripe grapes are grown and consumed especially in Mediterranean and Southeastern Anatolia region countries. Verjuice has a specific aroma, flavour and sour taste (Aminian, Massoompour, Sadeghalvaad, & Omrani, 2003; Stradley & Cook, 2000). It can be also called as “unripe grape juice”, “green juice” or “sour grape juice” in literatures (Hayoglu et al., 2009).

The composition of the grapes varies during ripening of the fruit and largely dependent on the soil structure, location and climates (Somogyi, Barret, & Hui, 1996).

Verjuice is obtained from unripe grapes and has the similar components to those found in the grape juice. The basic composition of verjuice includes water, carbohydrates (glucose and fructose), protein, calcium, phosphorus, iron, sodium, potassium, thiamine (B_1), riboflavin (B_2), niacin (B_3) and ascorbic acid (Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Publication, 2006). The amount of sugar, acids and flavour materials such as methyl anthranilate and other volatiles, tannins and colour substances determine the quality of the juice (Bates et al., 2001). Organic acids are essential determinants of fruit maturity. The main organic acid of the verjuice is tartaric acid. Tartaric acid can be found in free form or salt form of potassium or sodium bitartarates in juice (Somogyi et al., 1996). As grapes ripen, acid levels fall and sugar levels rise. Both sugar and acid are important. Higher sugar levels are associated with grape maturity (Soyer et al., 2003). In conjunction with this, acidity of the grapes influences the quality of the verjuice. If the acidity increases above 0.85 % of the regular level, the grape juice possesses a very sharp taste (Somogyi et al., 1996). It is also known that grapes are rich in polyphenols. Catechin, epicatechin, epicatechingallate, caftaric acid, coutaric acid (cis- and trans- form) are identified in the white grapes (Borbalan, Zorro, Guillen, & Barroso, 2003). These phenolic matters are also expected to be find in grape juice products (Dani et al., 2007). Additionally, chlorophyll and carotenes that are present in the grapes are responsible from color of the juice (Somogyi et al., 1996). Trans-resveratrol (3,5,4‘‘-trans-trihydroxy-stilbene), is an important polyphenol in the grapes, can be also found in verjuice and show antioxidant and anticarcinogenic effects.

Several studies showed that verjuice has various benefits on the human health, and it is a valuable juice for the human diet. Karapinar and Sengun (2007) studied the antimicrobial effect of the verjuice. They indicated that *Salmonella typhimurium* can be eliminated from the vegetable salads by using verjuice as a flavour agent. Flavonoids such as catechin and anthocyanin that are important antioxidant compounds are also detected in the verjuice composition (Nematbakhsh et al., 2013; Setorki, Asgary, Eidi, & Rohani, 2010). Similarly, Shojaee-Aliabadi et al. (2013) also stated that the verjuice extract can be used as a natural and inexpensive antioxidant. Verjuice is used as a traditional medicine by Iranian people. It provides a protection against cardiovascular disease, and has a blood pressure and lipid lowering properties (Aminian, Aminian, & Hoseinali, 2006; Setorki et al., 2010; Nematbakhsh et al., 2013). Other works reported anti atherosclerotic effectsand cholesterol and LDLs lowering effectsof verjuice (Setorki, Nazari, Asgary, Azadbakht, & Rafieian-Kopaei, 2011; Aminian et al., 2003; Mousa-Al-Reza, Ziba, Zakieh, rad Tania,

& Mohammad-Mahdi, 2011; ZibaeeNezhad, Mohammadi, Babaie Beigi, Mirzamohammadi, & Salehi, 2012). Additionally, verjuice can lower pH and alcohol content of wines and controls the technological problems (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2011).

2.6.4. Verjuice Powder

Verjuice powder is a new product developed in this Ph.D. thesis and has not been investigated in the literature yet. The verjuice is commonly consumed in the form of juice or concentrate and prepared seasonally in the household conditions. It has a limited shelf life because of the microbiological spoilage. However, the verjuice powder can be produced as an alternative form of souring agent with a longer shelf life to give aroma in meals, salads and snacks. The nutritional content and health benefits of verjuice also contribute the manufacturing of this alternative powder product. Additionally, powder form of verjuice ensures the availability of this seasonal product throughout the year in the markets.

2.6.5. Concentrated Verjuice

The concentrated verjuice can be defined as similar with the description of the concentrated grape juice given by Codex Alimentarius Commission (Codex Stan 83-1981). The concentrated grape juice is defined as "the unfermented product which is capable of fermentation after reconstitution, preserved exclusively by physical means, obtained from the grapes by the process of concentration. The product has a soluble grape solids content of not less than 30% (w/w) as determined by refractometer at 20 °C (Alimentarius, 1981a). The product may be turbid or clear, but is substantially free of crystals of salts of tartaric acid. The concentrated grape juice may be clarified and corrected for acidity" (Alimentarius, 1981a).

Concentrated verjuice has similar characteristics with the concentrated pomegranate juice. Pomegranate juice concentrate is widely used in salads and meals (Maskan, 2006). Thus, there is a potential for similar use of the verjuice concentrate. The total soluble solid content of pomegranate juice concentrate must be 65 °Brix (Meykon, 2018; Milnefruit, 2018). Therefore, the total soluble solid content of the concentrated

verjuice is required to be adjusted to 65 °Brix. The nutritive and health promoting components of the reconstituted verjuice may not be the same as freshly squeezed verjuice (section 2.6.3) depending on the applied evaporation process. There is no unadulterated verjuice concentrate, i.e. without any additives (sugar, olive oil or salt), sold in the market within our knowledge.

2.6.5.1. The Potential of Verjuice Products in the Market

Verjuice is produced from squeezing of unripe grapes and consumed locally in Mediterranean and Southeastern regions in Turkey and Neighbouring countries (Hayoglu et al., 2009). In this regions, verjuice or its concentrated form can be consumed as a sauce or beverage with the addition of some ingredients such as sugar, olive oil and salt. It is a savoury alternative to vinegar and lemon juice and used as a sauce form in traditional courses, salads and snacks to give flavour, and mixed in several alcoholic beverages (Hildebrandt & Matchuk, 2002; Stradley & Cook, 2000). It can be also used as fermented form based on consumption habits in certain regions (Anon, 2003). Additionally, Karapinar and Sengun (2007) reported the use of verjuice as a comforting drink after consumption of desserts. Verjuice can also be used as a medicinal purposes by Iranian people (Setorki et al., 2010). Because of the special aroma and health benefits of verjuice, it can be produced and consumed in different forms, i.e. pasteurized, concentrated or powder form.

There are different uses and recipes for verjuice in different cuisines. These products are called as sour grape juice (koruk ekşisi) and sour grape sauce (koruk ekşisi sosu) (Table 2.15). Generally, after adding some additives (sugar, salt, olive oil etc.) to the juice, it can be used as a beverage (sour grape juice), or as a sauce (sour grape sauce) to give aroma in meals and salads. In the preparation of sour grape juice, clear unripe grapes are firstly squeezed by a fruit juice extractor (or manually), and then filtered to remove cloudiness in the juice. Sugar and water can be added to the freshly squeezed verjuice to decrease severe acidity of the product. This sour grape juice can be also concentrated by boiling in an open pan and then reconstituted before consumption. If the verjuice is used as a sour grape sauce in meals, the desired amount of olive oil and salt are added to the freshly squeezed verjuice. Alternatively, the verjuice can be concentrated

by boiling, and then oil and salt can be added to the concentrate. Sour grape juice and sour grape sauce are demonstrated in Figure 2.10.

In this Ph.D thesis, verjuice and its products (verjuice powder and concentrated verjuice) are prepared without any additives. Therefore, these verjuice products are going to be sold as gourmet products. Specialty foods are generally considered unique and high-value food items made in smaller quantities and from high-quality ingredients. The gourmet products are fancier, more upscale and more desirable.

Table 2.15. The main ingredients in verjuice products sold in the market

Sour grape juice (Koruk ekşisi/Koruk şerbeti)	**Sour grape sauce (Koruk ekşisi sosu)**
Unripe grapes	Unripe grapes
Water	Salt (desired amount)
Sugar (1:4)	Olive oil (desired amount)

a) b)

Figure 2.10. Sour grape juice (a) and sour grape sauce (b)

CHAPTER 3

VERJUICE PASTEURIZATION BY ULTRAVIOLET IRRADIATION (UV-C) TREATMENT

Fruit juice consumption has been rising in the last decades because of their nutritional properties (Bates et al., 2001). Verjuice is an unfermented grape juice which is obtained by squeezing of unripe table grapes. It is usually produced in a household conditions. Thus, it is prone to microbial spoilage caused by fungi naturally grown in grapes. Thermal pasteurization is conventionally used to destroy spoilage and pathogenic microorganisms and inactivate enzymes in the fruit juices (Azhu Valappil et al., 2009; Igual, Garcia-Martinez, Camacho, & Martinez-Navarrete, 2010; Pathanibul, Taylor, Davidson, & Harte, 2009). It is the most common preservation method applied between 60 and 100 °C for a few seconds for fruit juice having a pH lower than 4.5 (Rivas et al., 2006). According to Food and Drug Administration (FDA) regulation mandated by Hazard Analysis Critical Control Point (HACCP) programme, "5-log reduction in the number of target microorganism is found to be necessary for fruit juice pasteurization" (U.S. FDA, 2001). However, thermal pasteurization applied at high temperatures causes several sensorial and nutritional quality problems such as loss of flavour and taste, degradation of nutrients and undesirable browning reactions (Garde-Cerdan et al., 2007; Walkling-Ribeiro et al., 2008). In order to avoid these quality losses, nonthermal processes are developed as an alternative preservation method to obtain more fresh-like, wholesome and healthful fruit juices (Noci et al., 2008). UV-C irradiation (short wave ultraviolet light) which is one of the nonthermal technologies has a lethal impact on microorganisms (Bintsis et al., 2000). FDA approved UV-C irradiation as an alternative pasteurization processes for fruit juices (U.S. FDA, 2000).

The objective of this chapter was to determine suitable pasteurization conditions for verjuice to provide 5-log reduction of target microorganism, i.e. *S. cerevisiae* (NRRL Y-139), using UV-C irradiation, mild heating and the combination of UV-C and mild heating processes. Firstly, *S. cerevisiae* was acid adapted and inoculated into freshly squeezed verjuice. *S. cerevisiae* was inactivated by exposing to UV-C irradiation, mild heating and combination treatments by means of a laboratory-scale continuous flow UV

reactor system. Kinetic parameters of microbial inactivation were calculated by using a suitable model. Processing conditions for UV-C irradiation were determined based on 5-log reduction of *S. cerevisiae* and then used for the shelf life study of verjuice in the following chapter.

3.1. Materials and Methods

3.1.1. Raw material: Koruk (Unripe Grape)

Unripe grapes (*Vitis vinifera L*., var. Yediveren) that is named as “Koruk” in Turkey were purchased from vineyards in Urla region of Izmir. The ripening index and harvesting time of the grapes was determined from the maturation index given in Equation 3.1 (Palomo et al., 2007).

$$\text{Maturation index} = °\text{Brix} (\%)/\text{acidity} (\%) \quad (3.1)$$

Unripe grapes were first de-stemmed, washed with 5% grape vinegar solution (Kukre Gida A.S., Turkey) (containing 0.25% w/v acetic acid), and deionized water (2L/kg grapes) to remove any foreign particles such as leaves, soil from the surface of the grapes and to reduce initial microbial load of the grape. After washing steps, surface of the koruks were dried in the oven for a short time and stored at the freezer (-20 °C) until use.

3.1.2. Verjuice Preparation

Verjuice was obtained by following defrosting, pressing and de-tartarization steps (Figure 3.1). A household table top fruit juice extractor (Arçelik, Robolio, İstanbul) was used to crash the unripe grapes after defrosting step and strained by means of a sieve. The freshly squeezed verjuice was stored in the refrigerator (4 °C) for 24 h to precipitate the tartarate particles. Then, the clear verjuice was obtained after removing of sediments.

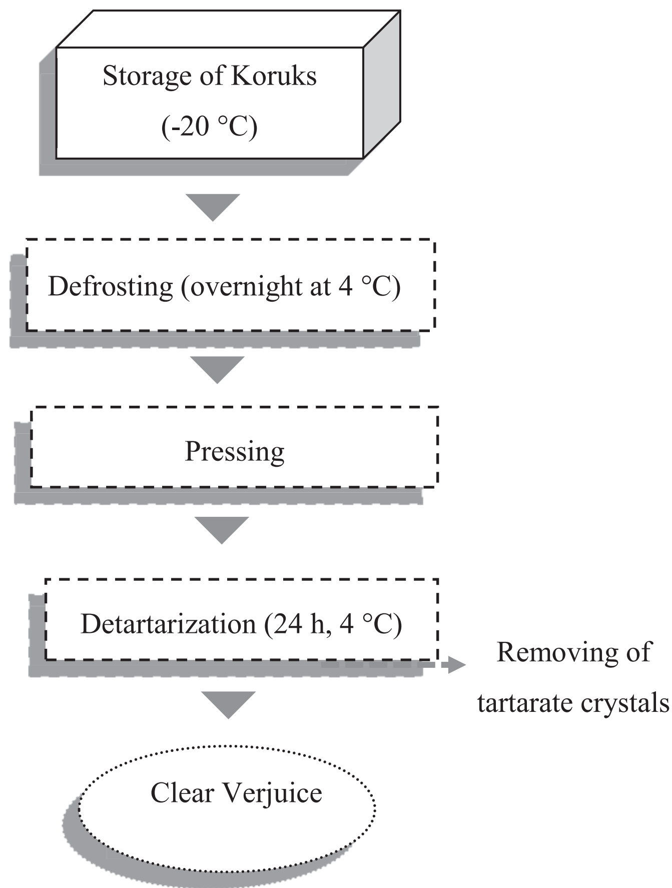


Figure 3.1. Flow diagram of verjuice production

3.1.3. Verjuice Characterization

Freshly squeezed verjuice was characterized prior to the microbial inactivation studies. For this purpose, pH, brix, titrable acidity, absorption coefficient, turbidity and color parameters were measured. The details of these techniques will be discussed in Chapter 4. Briefly, pH and total soluble solid (°Brix) of verjuice were measured by a bench top pH meter (Inolab 7310, WTW series, Germany) and a hand-held refractometer (Pocket refractometer PAL-1, Atago, Co, Ltd, Tokyo Tech., Japan) at room temperature (20 °C). Titratable acidity (%) was determined according to the method of AOAC (1984). Carry 100 UV-Visible Spectrophotometer (Varian Inc., CA, USA) set at 254 nm and a turbidimeter (Model 2100AN IS, HACH Company, USA) was used to measure absorption coefficient (cm^{-1}) and turbidity (NTU) values of verjuice. CIE color parameters (L*, a* and b*) were also determined by using Konica Minolta CR 400 Chromometer (Konica Inc, Japan).

3.1.4. Microbiological Analysis

3.1.4.1. Background Microflora of Verjuice

Background flora of freshly squeezed verjuice was determined prior to microbiological inactivation studies. For this purpose, the numbers of total mesophilic aerobic, yeasts and moulds, and total coliforms were counted by spread plating on the Plate Count Agar (PCA), the Potato Dextrose Agar (PDA) and Violet Red Bile Agar (VRBA) plates, respectively. PCA (Merck, Darmstadt, Germany), PDA (Merck, Darmstadt, Germany) and VRBA (Merck, Darmstadt, Germany) plates were incubated at 30 °C for 48 hours, at 25 °C for 2-5 days and at 37 °C for 24 h, respectively. Appropriate dilutions were made with 0.1% peptone water (Merck, Darmstadt, Germany). Results were expressed as CFU/mL.

3.1.4.2. Target Microorganism, Sample Inoculation and Enumeration

Verjuice is an acidic product (pH around 2.6-2.7) and the main spoilage microflora is dominated by yeast and moulds. These microorganisms are found in unripe grapes in vineyards, transferred into verjuice during pressing and spoil the juice if inefficient washing step is applied.

Verjuice was initially fermented in order to define the dominating strain with spoilage potential that would be used in further inoculation studies. For this purpose, freshly squeezed verjuice was obtained from the unwashed berries (its surface was not disinfected) to keep the natural microbial flora of the grapes. This juice was placed in a shaker (Thermo Electron Corp., Ohio, USA) and shook at 200 rpm and 30 °C for a couple of days. At the end of natural fermentation trials, several moulds and yeasts species were detected in the verjuice samples. The cells were counted on PDA and morphology of the cells were examined by using a phase contrast microscope (Olympus CX31, New York, USA) (Figure A.1). On the basis of the staining and microscopic results, yeasts were found to be the main microorganisms causing spoilage of verjuice. Thus, *Saccharomyces cerevisiae* (NRRL Y-139) was selected as the target microorganism in verjuice to evaluate the efficiency of processes. Besides *S. cerevisiae* is the heat resistant

microorganism, Gabriel (2012) suggested an application of 5D concept for the spoilage microorganism that shows the greatest growth rate in storage. So, 5 log CFU/mL reduction of *S. cerevisiae* in verjuice was aimed to mimic the worst scenario condition in verjuice.

Stock cultures of *S. cerevisiae* (NRRL Y-139) containing 25% glycerol was stored at the freezer (-80 °C) until use. Cultivation of *S. cerevisiae* strains was done by using Yeast extract-Peptone-Dextrose (YPD) Broth (prepared by manually according to literatures instructions: 10 g yeast extract, 20 g peptone, 20 g dextrose per 1 L distilled water). It is important to make sure that *S. cerevisiae* (NRRL Y-139) cells can survive in the freshly squeezed verjuice before UV-C and heat treatment processes. 100 µl of cells from the stocks (-80°C) were initially cultivated in 100 mL YPD broth (pH 6.55) and incubated at 30 °C and 200 rpm for 24 hours. 500 µL of culture from the stock solution was directly inoculated to 30 mL of verjuice and incubated at the same conditions (30 °C, 200 rpm for 24 hours). Sampling was done at different time intervals during incubation. It was observed that the cells were rapidly died in the high acidic juice conditions. The number of the *S. cerevisiae* in verjuice was reduced from initial load of 5.89±0.06 log CFU/mL to 4.49±0.01 log CFU/mL within six hours (Figure 3.2). Thus, several acidification conditions were tried in order to provide the viability of the cells in highly acidic environment of verjuice (Table A.1 in Appendix A). The viable *S. cerevisiae* cells in the verjuice (after waiting for 72 h) were transferred to the PDA slants (acidified to pH 3.5 with 10% tartaric acid) and stored at 4 °C until used again in the acid adaptation experiments.

S. cerevisiae cells were adapted to high acidic conditions by reducing pH of the YPD broth. *S. cerevisiae* (NRRL Y-139) culture in the PDA slants was cultivated to 10 mL YPD broth and incubated for 24 h at 30 °C and used for the acid adaptation study. The most suitable acid adaptation procedure in Table A.1 was selected (6th condition). In the first step of this procedure, 100 µL of cultivated *S. cerevisiae* strains was inoculated into 100 mL YPD broth (pH 3.5) and incubated in an orbital shaker (Thermo Electron Corp., Ohio, USA) for 24 hours at 200 rpm and 30 °C. In the second step, 1 mL of enriched cells in YPD broth at pH 3.5 were transferred to YPD broth having a pH of 2.7 (i.e. the pH that was close to pH of the verjuice) and incubated in the shaker for 48 h at the same conditions (30 °C & 200 rpm). Finally, 1 mL from these acid adapted *S. cerevisiae* cells were inoculated into verjuice (30 mL) and enumeration was performed using Potato Dextrose Agar (PDA, Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10%

tartaric acid (Merck, Darmstadt, Germany) after incubated 48 h at 30 °C. The number of the acid adapted yeasts was reached to approx. 10^6 CFU/mL. Additionally, it was clearly seen that acid adapted yeast cells in verjuice were not reduced significantly (Figure 3.2) and these viable cells were used in further inactivation studies.

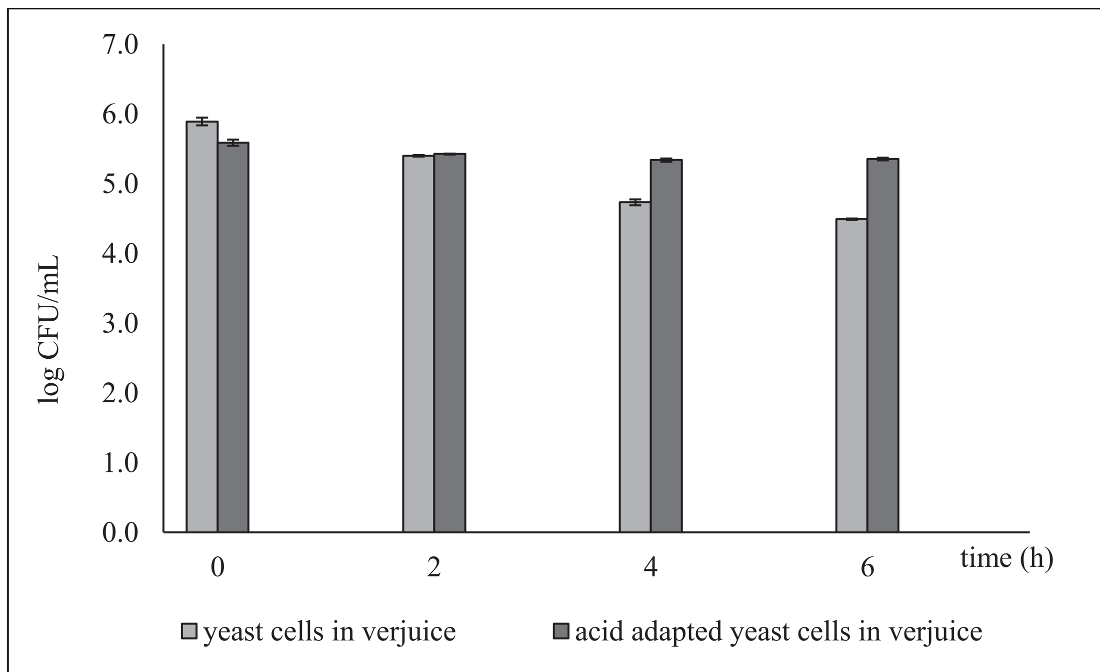


Figure 3.2. Viability of *S. cerevisiae* (NRRL Y-139) in freshly squeezed verjuice before and after acid adaptation (light grey: cells not acid adapted, dark grey: acid adapted cells)

A loopful of acid-adapted culture from the PDA slant was first inoculated into 100 mL YPD broth (prepared in the same way as in the previous acid adaptation procedure) for enrichment. At the end of the enrichment period, the number of acid adapted yeast cells was reached to approx. 10^6 CFU/mL. The verjuice samples were directly inoculated with these cells to have an initial load (low and high level) of around 10^4 or 10^6 CFU/mL. Inoculated verjuice samples were subjected to UV-C irradiation, mild heating, UV-C combined with mild heat treatment, as well as conventional thermal pasteurization. Conventional thermal pasteurization was explained in detailed in Chapter 4. To enumerate the viable microorganisms in the treated and untreated samples, appropriate dilutions were made with 0.1% peptone water (Merck, Darmstadt, Germany) and surface plated in duplicate on Potato Dextrose Agar plates acidified to pH 3.5 with 10% tartaric acid. All of the plates were incubated at 30 °C for 48 h. Results were expressed as colony-forming units per milliliter (CFU/mL).

3.1.5. Experimental Procedure

Figure 3.3 shows the flow diagram of the studies conducted in this chapter. Briefly, clear verjuice samples were inoculated with acid adapted *S. cerevisiae* (NRRL Y-139) cells and exposed to further treatments such as UV-C irradiation (UV), mild heating (MH), UV-C combined with mild heat treatment (UV+MH). All treatments were conducted by using continuous flow UV-C reactor system, i.e. UV was applied without any heating, MH was applied to verjuice samples by using the same system without turning on UV lamps, and combined UV+MH was applied at mild temperatures with turning on UV lamps. The effects of treatments on the inactivation of *S. cerevisiae* in verjuice were then evaluated based on 5-log reduction of target microorganisms required for pasteurization of verjuice. Additionally, linear and nonlinear models were applied to inactivation kinetics data. Synergistic or additive effect of combined processes (UV+MH) was evaluated using the best fitted model parameters. The experiment conditions determined in this chapter used in the studies outlined in the next chapter (Chapter 4) in which verjuice was pasteurized with the best nonthermal processing conditions.

3.1.6. UV-C Irradiation of Verjuice (UV)

Freshly squeezed verjuice samples inoculated with acid adapted *S. cerevisiae* (NRRL Y-139) strains were exposed to UV-C irradiation by using Laboratory Scale Continuous Flow UV-C reactor system (Figure 3.4). The main components of the system are; a glass sample tank equipped with a water circulation jacket, stainless steel UV reactor that is composed of an annular type quartz glasses at the centre, seven UV-C lamp emitting light at 254 nm, one of them is centred in the middle, others are placed around the annular quartz glasses, a peristaltic pump that provides circulation of the juice through the system and silicon tubing to provide flow of the juice. The water around the glass sample tank was circulated by means of a water bath (Haake DL30, Thermo Electron Corp., Karlsruhe, Germany) equipped with a cooler (Haake EK45, Thermo Electron Corp., Karlsruhe, Germany). The four hundred mililiters verjuice was circulated in the annular flow region where a path length is 5 mm. The temperature of juice at the outlet of the UV system and inside the sample tank was checked by a K-type thermocouple (CEM DT-8891E, Shenzhen, China).

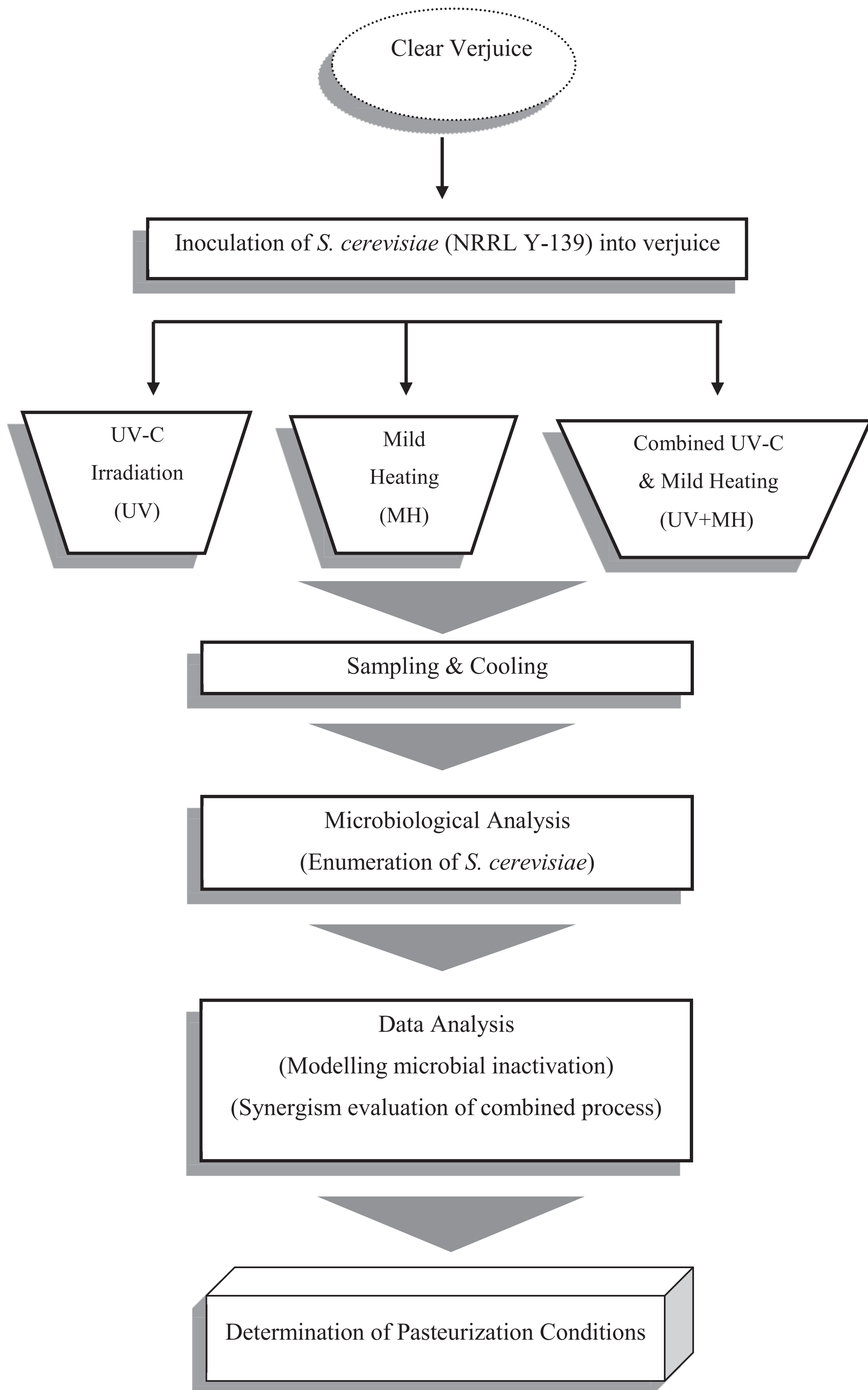


Figure 3.3. The flow diagram of inactivation studies conducted in Chapter 3

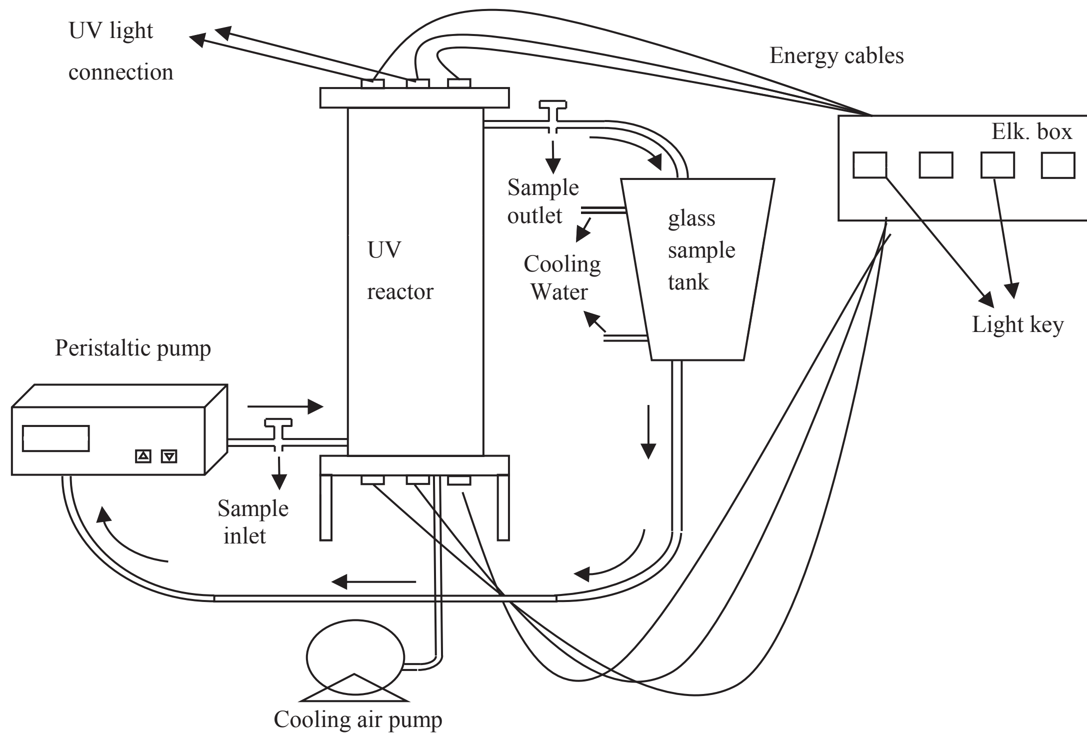


Figure 3.4. Laboratory Scale Continuous Flow UV-C Reactor System

Time necessary to complete one cycle of four hundred mL verjuice in the UV-C system was recorded and calculated from Equation 3.6 where t_1 is the time for the verjuice to reach the UV-C reactor inlet, t_2 is the UV exposure time throughout the annular quartz tube, t_3 is the time necessary for returning of verjuice to the sample tank after UV irradiation and t_4 is holding time to complete one cycle of remained verjuice in the sample tank. The whole flow volume was collected in the glass sample tank at the end of one total cycle time during UV operation. Then, the next cycle was started (Unluturk & Atilgan, 2014). Total UV exposure time of verjuice for 8 cycles was also calculated from Equation 3.7.

$$t_{total} = t_1 + t_2 + t_3 + t_4 \tag{3.6}$$

$$t_{uv} = 8\ (cycling\ time) * t_2 \tag{3.7}$$

Before the UV-C process, the freshly squeezed verjuice samples were inoculated with acid adapted *S. cerevisiae* cells with the two different initial microbial load, i.e. high

level (6.36±0.04 log CFU/mL) and low level (4.55±0.09 log CFU/mL), were used to evaluate the effect of the initial load on the performance of the system.

The inoculated verjuice was circulated through the reactor using a lamp configuration which was designated as configuration I (four lamps on). In this configuration one lamp placed in the centre and other three lamps located around the reactor were turned on throughout the UV processing (Figure 3.5). In the UV processing, verjuice was pumped through the UV-C system at 16 °C (T_{juice}), and water at 10 °C ($T_{water\ circulation}$) was circulated around the holding tank during processing (cold pasteurization condition). The juice was circulated eight times in the system at a flow rate of 3.80 mL/s (70 rpm pump frequency). The UV experiments were repeated three times and sampling was performed after each cycle. The numbers of *S. cerevisiae* colonies in the verjuice samples were counted by spread plating on PDA plates. The survival curve of microorganisms was drawn by plotting log (N/N_0) reductions versus time (s), where N is the residual microbial load (CFU/mL) at a given time and N_0 is the initial microbial load (CFU/mL).

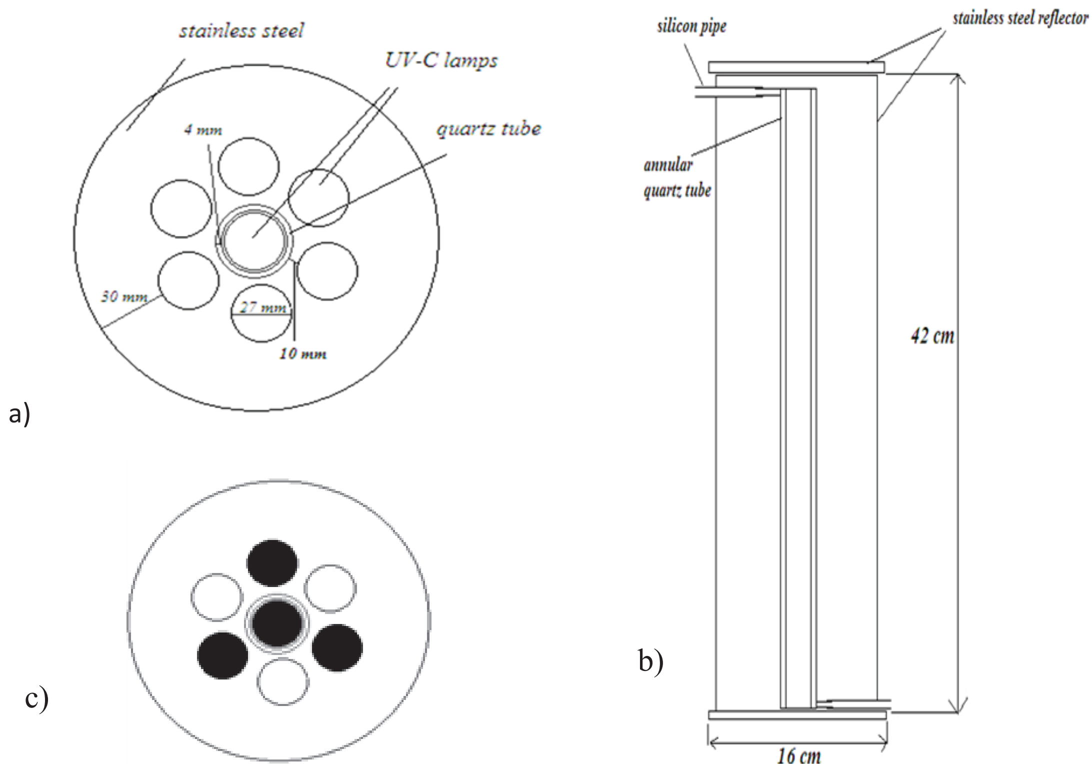


Figure 3.5. Dimensions of the UV-C reactor (a & b) and arrangements of the 7 UV-C lamps in the processes (c: Black circle: open lamp, empty circle: closed lamp)

Additionally, some optical properties e.g. absorption coefficient (cm^{-1}), color parameters (L*, a*, b*) and turbidity (NTU) of untreated verjuice samples were also measured to evaluate their UV-C light absorbing characteristics.

3.1.7. Mild Heating (MH)

Two different mild heat treatments (MH1 & MH2) were applied by means of the continuous flow UV reactor system (Figure 3.4). All the UV lamps in the system were turned off. Therefore, the effects of thermal processes on acid adapted *S. cerevisiae* (NRRL Y-139) strains in verjuice samples were determined only at two different mild temperatures.

For this purpose, continuous flow UV reactor system was connected with a water bath ($T_{water\ circulation}$) that was adjusted to 55 °C (MH1) and 60 °C (MH2), respectively. Heated water was circulated around the UV-C reactor to keep juice at selected temperature, i.e. around 50 °C. For this purpose, four hundred mL of verjuice was initially pre-heated to 50 °C (T_{juice}). Acid adapted *S. cerevisiae* culture was then inoculated to juice at the initial load of approx. 10^5 CFU/mL. The inoculated verjuice was circulated 8 times in the system at a flow rate of 3.80 ml/s (70 rpm pump frequency). Experiments were carried out as 3 replicates. Sampling was performed after each cycle. The number of *S. cerevisiae* colonies in the verjuice samples before and after the treatments were counted by spread plating on PDA plates.

3.1.8. UV-C Irradiation Assisted with Mild Heating (UV+MH)

UV-C irradiation was combined with two different mild heat treatments (UV+MH1 & UV+MH2) to study the effect of “hurdle technology” on the microbiological quality of verjuice samples. The aim of the hurdle concept was to increase the logarithmic reduction of *S. cerevisiae* (NRRL Y-139) in verjuice.

Experiments were carried out using the same UV-C processing and mild heating conditions. Continuous flow UV reactor system (Figure 3.4) was connected with a water bath adjusted to 55 °C (UV+MH1) and 60 °C (UV+MH2), respectively. Four hundred mL of pre-heated juice (T_{juice}: 50 °C) was inoculated with acid adapted *S. cerevisiae* with an initial load of approx. 10^5 CFU/mL and circulated 8 times throughout the heated

system at selected temperatures with a flow rate of 3.80 ml/s. The temperature of the verjuice within the sample tank and at the outlet of the UV-C system was checked by K-type thermocouple (CEMDT-8891E, Shenzhen, China) during processes. Experiments were carried out as 3 replicates. Sampling was performed after each cycle. The number of *S. cerevisiae* colonies in the verjuice samples before and after the treatments were counted by spread plating on PDA plates.

3.1.9. UV dose calculation: Actinometrical Study

The applied UV dose in the continuous flow UV-C reactor system was measured by using iodide/iodate actinometrical method (Kaya et al., 2015; Rahn, 1997). According to this method, potassium iodide/iodate actinometer solution was prepared by dissolving of 0.6 M potassium iodide (Merck, Germany) and 0.1 M of potassium iodate (Merck, Germany) solutions in 0.01 M borate buffer (pH 9.25) (Merck, Germany). The method is based on measuring of the absorbance of triiodide formation at 352 nm spectrometrically after the linear conversion of iodide (I^-) to triiodide (I_3^-) by irradiation. The number of photons absorbed by the sample after irradiation was calculated from the moles of triiodide formation (Rahn, 1997).

In this study, 400 mL actinometer buffer was pumped through the UV-C system at the same conditions as the verjuice sample (8 cycles, 3.80 ml/s flow rate & lamp configuration I). The increase in absorbance (352 nm) per each cycle was measured by using a UV-visible spectrophotometer (Carry 100, Varian Inc., CA, USA) at the outlet of the reactor. At the end of the several calculations, UV dose was estimated per each cycle in the system.

Initially, concentration of iodide (KI) solution was measured at 300 nm before UV-C experiments and initial iodide concentration was calculated from the Equation 3.8.

$$[c_{iodide}](M) = \left. \frac{OD}{1.061 * l} \right|_{@300\ nm} \tag{3.8}$$

where 1.061 ($M^{-1}*cm^{-1}$) is referred to the extinction coefficient (ε) and l was the path length which is 1 cm (the width of the quartz cuvette used in spectrophotometer). Then, quantum yield (Φ) which is defined as the rate of photochemical reaction (the ratio of

number of absorbed molecules to the number of photons absorbed per unit time) was determined based on temperature dependent formulation given in the Equation 3.9. After UV-C irradiation, concentration and moles of triiodide were also determined from the Equations 3.10 and 3.11, respectively where 26400 (M^{-1}*cm^{-1}) is the molar extinction coefficient of triiodide formed in a 1 cm path length and sample volume in the annular reactor (V_{sample}) is 219.8 mL. Then, number of Einsteins of photons absorbed by sample was also calculated from Equation 3.12. Finally, incident joule (J) was determined from Equation 3.13.

$$\Phi = 0.75\,[1 + 0.02\,(T - 20.7)][1 + 0.23\,(c_{iodide} - 0.577)] \quad (3.9)$$

$$[c_{triiodide}](M) = \left.\frac{OD}{26400\,\frac{1}{M\ cm} * l}\right|_{@352\ nm} \quad (3.10)$$

$$n_{triiodide} = c_{triiodide} * V_{sample} \quad (3.11)$$

$$E\left(\frac{einsteins}{L}\right) = \frac{\Delta OD_{352\ nm} * V_{sample}}{26400 * \Phi} \quad (3.12)$$

$$Incident\,joules = \frac{4.72 * 10^{5}\,J}{Einstein\,photons} * E\left(\frac{Einstein}{L}\right) \quad (3.13)$$

After actinometrical UV dose calculations, UV dose absorbed by verjuice was determined by using Beer-Lambert Law (Mozowitz, 1950; Unluturk & Atilgan, 2015). Firstly incident intensity (I_o) was obtained from Equation 3.14 dividing incident joule to the surface area of the annular reactor (area: 879.65 cm^2). Then, average UV intensity (I_{avg}) absorbed by verjuice per each cycle was calculated from Beer-Lambert Law given in Equation 3.15 where A_e is the absorption coefficient of verjuice (25.8 cm^{-1}) and L is the path length of the annular reactor (0.5 cm). UV dose absorbed by verjuice per each cycle in the system was estimated as "mJ/cm^2" where t is UV exposure time (s) from Equation 3.16 and as "mJ/mL" from the Equation 3.17.

$$I_0\,(\frac{W}{cm^2}) = \ incident\ joule\ /area\ (cm^2) \tag{3.14}$$

$$I_{avg}(\frac{W}{cm^2}) = \ I_0\ * (1 - e^{-Ae*l})/Ae * l \tag{3.15}$$

$$UV\ Dose\ \left(\frac{mJ}{cm^2}\right) = \ I_{avg} * t \tag{3.16}$$

$$UV\ dose\ \left(\frac{mJ}{mL}\right) = \frac{Uv\ dose\left(\frac{mJ}{cm^2}\right) * annular\ area\ (cm^2)}{V_{annular}\ (mL)} \tag{3.17}$$

3.1.10. Modelling of UV-C Inactivation Kinetics of *S. cerevisiae* in verjuice

A model can be defined in as "the description of a system, theory, or phenomenon that accounts for its known or inferred properties and may be used for further study of its characteristics" (McMeekin et al., 2008). Microbial inactivation during food processing can be described with the concept of "predictive microbiology" based on several mathematical models (Valdramidis, Bernaerts, Van Impe, & Geeraerd, 2005). These models should provide to produce stable and safe foods with a required level of inactivation in suitable processing conditions (Manas & Pagan, 2005).

Generally, microorganisms in foods exposed by thermal and nonthermal treatments can exhibit eight different inactivation behaviors, such as linear, linear with tailing, sigmoidal-like, linear with shoulder, and biphasic types of these curves shown in Figure 3.6 (Geeraerd, Valdramis, & Van Impe, 2005). These inactivation behaviors are depended on several factors such as process parameters (time, temperature, dose etc.), types of microorganisms (bacteria, yeasts, molds etc.) and food properties (pH, water activity, medium composition etc.) (Manas & Pagan, 2005).

The inactivation kinetics of a UV dose response curve in this study can be explained by using linear and non-linear inactivation kinetic models (Figure 3.6).

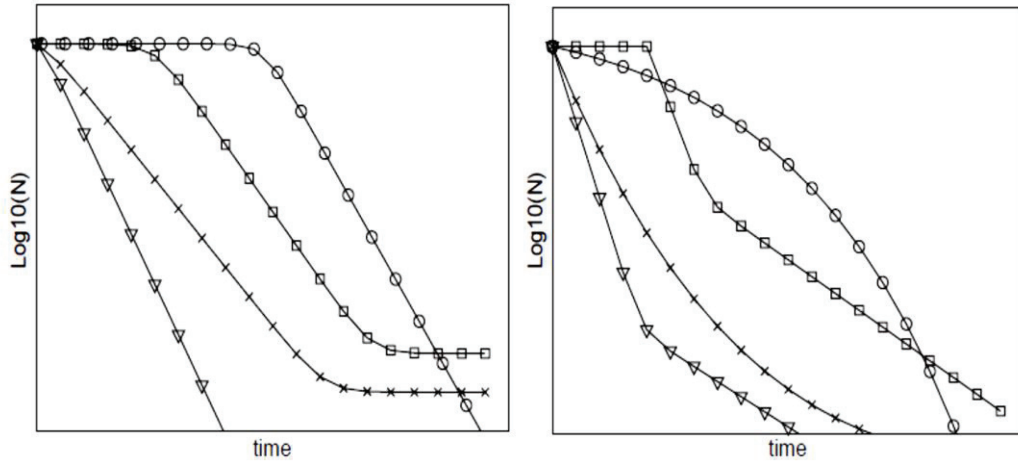


Figure 3.6. Several types of microbial inactivation curves (Left graph; linear: Δ, linear with tailing: x, sigmoidal-like: □, linear with a preceding shoulder: o) (Right graph; biphasic: Δ, concave: x, biphasic with a shoulder: □, and convex: o) (Source: Geeraerd et al., 2005)

Thermal inactivation of microorganisms was conveniently explained by the first-order kinetics with a linear model shown in Equation 3.18 (Chick, 1908). According to the model, survival number of microorganisms is linearly related with treatment time of heating (Manas & Pagan, 2005). This equation could be converted into log-linear model in Equation 3.19 to calculate decimal reduction time (D) of microorganisms (Van Boekel, 2002).

$$N(t) = No * e^{-kt} \tag{3.18}$$

$$\log\left(\frac{N}{No}\right) = -\frac{t}{D} \tag{3.19}$$

where N is the number of survival count (CFU/mL) after treatments at time t (s) time, N_0 is the initial microbial count (CFU/mL) before treatments and k is the inactivation rate constant (s^{-1}). D value that is the time needed for 90% inactivation of the cells was calculated as second (D = 2.303/k). Inactivation kinetic parameter (k), RMSE and R^2 values were identified by GlnaFiT model-fitting software program (KU Leuven, Leuven, Belgium) for each processes.

Microbial inactivation curves do not always show a linear trend. The curve might have upward or downward concavity that caused by "shoulder" or "tailing" effect depending on the conditions (Mafart, Couvert, Gaillard, & Leguerinel, 2002). Karel and

Lund (2003) indicated that microorganisms exposed to UV-C irradiation generally show this behavior. Shoulder region (injury phase) may occur in the non-lethal UV dosage which is inert to the cells at the initial of the process; cells were exponentially died over this non-lethal dosage (linear section); and then tailing region may form in the case of a presence of very resistant strain of organisms or suspended solids at the end of the process (Figure 3.7). According to the presence of shoulder, tail or both of them in the curve, different models could be adjusted to the inactivation data of the microorganisms.

The survival curves do not show tail but rather shoulder behavior in some cases. Thus, log-linear regression plus shoulder model described by Geeraerd, Herremans, and Van Impe (2000) can be used, which is described by the Equation 3.20:

$$N_t = N_0 * e^{-kmax * t} \left(\frac{e^{kmax * SI}}{1 + \left(e^{kmax * SI} - 1\right) e^{-kmax * t}} \right) \tag{3.20}$$

where N_0 and N_t represent number of survival counts before the treatments and after time t, one of the model kinetics parameters SI means shoulder length time before exponential inactivation begins and the other one k_{max} is inactivation rate constant of exponential death region. In order to evaluate the fitting of the model to the data in this study, kinetic parameters as well as RMSE values of the model were calculated by using GlnaFiT program and compared with other models.

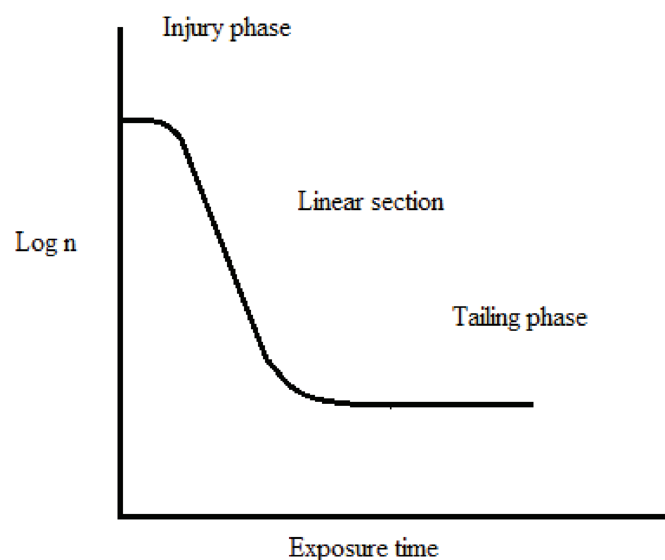


Figure 3.7. Typical survival curve of UV exposed microorganisms (log n: number of survival microorganisms) (Source: Karel & Lund, 2003)

Shoulder effect does not always appear in the inactivation curve of microorganisms. Log linear plus tail model developed by Geeraerd et al. (2000) can be used in the case of inactivation curve having only tail region without shoulder. It is described in Equation 3.21:

$$N_t = (N_0 - N_{res}) * e^{-kmax * t} + N_{res} \quad (3.21)$$

where N_0 and N_t are the number of survival microbial count before the treatments, and after time t, respectively and kinetic parameters N_{res} that is the residual number of subpopulations at the end of the treatment and k_{max} inactivation rate of exponential region.

The microbial inactivation is basically described as a homogenous process and microorganisms were inactivated linearly based on the first order kinetic model. However, the kinetic model might be heterogeneous due to the different stability of the each microbial cell in a population even if it composed of same species. Inactivation curve has both shoulder (downward concavity) and tailing phases (upward concavity) after logarithmic survival count of microorganisms is plotted versus time. In such conditions, non-linear model described by Weibull distribution could be used (Mafart et al., 2002; Manas & Pagan, 2005; Peleg & Penchina, 2000; Van Boekel, 2002). The equation of the Weibull model developed by Mafart et al. (2002) was shown in Equation 3.22. Besides general terms N (survival counts after treatment at time t), No (initial microbial number before treatments) and t (exposure time), there are two kinetic parameters in Weibull model; p is called shape parameter and describes downward concavity (p>1) or upward concavity (p<1) of the curve (if p=1, curve is linear). δ called scale parameter and describes the first decimal reduction time of survival cells similar to D value (Mafart et al., 2002).

$$\frac{N}{No} = 10^{-(\frac{t}{\delta})^p} \quad (3.22)$$

For all log-linear and non log-linear models used in this study, the time needed for 5-log reduction of the survival microorganisms (5D) were calculated from the t value where N/N_0 was equal to 10^{-5}.

3.1.10.1. Calculation of Synergistic Effect of UV-C Irradiation and Mild Heating

The synergistic lethal effects of UV-C irradiation and mild heat treatments (UV+MH) were calculated according to Equation 3.24 (Gayan et al., 2012).

5D values were selected since 5 log reductions (99.999%) of the most resistant microorganisms in the juice were mandatory requirement by FDA. Theoretical $5D_{UV\text{-}+MH}$ values were calculated by considering the individual contribution of UV-C ($5D_{UV}$) and mild heating ($5D_{MH}$) processes (Equation 3.23). $5D_{UV}$ and $5D_{MH}$ values in Equation 3.23 were found using experimental data and the best fitted inactivation model. Experimental $5D_{UV\text{-}+MH}$ values were calculated and compared with the theoretical ones for prediction of the synergistic effect. If the inactivation effect of hurdle process (UV+MH) was higher than the individual inactivation effect of each process, the process would be synergistic. Otherwise, the hurdle process would be additive. Additive effect can be predicted from Equation 3.25 (Raso, Pagan, Condon, & Sala, 1998).

$$Theoretical\ 5D_{UV+MH} = \frac{(5D_{MH} * 5D_{UV})}{(5D_{MH} + 5D_{UV})} \quad (3.23)$$

$$Synergism\ (\%) = \frac{theoretical\ 5D_{UV+\ MH} - experimental\ 5D_{UV+\ MH}}{theoretical\ 5D_{UV+MH}} * 100 \quad (3.24)$$

$$D_{UV+MH} = 1/D_{UV} + 1/D_{MH} \quad (3.26)$$

3.1.11. Data Analysis

All results were expressed as their means and standard deviations. Inactivation kinetic parameters were determined by plotting the logarithm of survival counts (log CFU/mL) of *S. cerevisiae* NRRL Y-139 versus exposure time (s). Regression analysis was applied by using commercial spread sheet (Microsoft Excel, Redmond, WA, USA). One-way analysis of variance (ANOVA) were carried out to determine how significantly the independent variables (processes) affect the dependent variables (logarithmic reductions of

S. cerevisiae NRRL Y-139) by using the Minitab 16 software program (Minitab Inc., State College, PA, USA). Several log-linear and non-log-linear models (log-linear plus shoulder, log linear plus tail and Weibull model) were applied to these data. In order to determine goodness of the fits and overfitting of the log-linear and non-log-linear models, the root mean square error (RMSE), bias factor (B_f), accuracy factor (A_f), the Akaike information criterion (AIC) and the Bayesian Schwarz criterion (BIC) values were compared. Inactivation kinetic parameters, RMSE values of each model were predicted by GlnaFiT model-fitting software program (KU Leuven, Leuven, Belgium). All processes were repeated three times. Root Mean Sum of Squared Errors (RMSE) is calculated from the Equation 3.26 where "cal" and "exp" terms express calculation and experimental data, n-k is the number of degrees of freedom i.e., the number of data points n minus the number of degrees of freedom k (parameters and initial values) used (Geeraerd et al., 2005). Bias factor (B_f) and accuracy factor (A_f) were calculated based on the Equations 3.27 and 3.28 given by Ross (1996) to enable comparison of growth models with each other as well as with observations. Bias factor measures the systematic prediction of the observed data; accuracy factor measures the difference between observed and predicted data (Amazquita, Kan-King-Yu, & Le Marc, 2011). Ideally, perfect agreement between the observed and predicted data would lead to a bias factor and accuracy factor of 1. If A_f, B_f>1 or A_f, B_f<1, it means that the model overestimates or underestimates the observations, respectively.

$$RMSE = \sqrt{\frac{1}{n-k} \sum \left[\left(\log \frac{N}{N_0} \right)_{cal} - \left(\log \frac{N}{N_0} \right)_{exp} \right]^2} \tag{3.26}$$

$$Bf = 10^{\left(\sum \frac{\log \left(\frac{predicted}{observed} \right)}{n} \right)} \tag{3.27}$$

$$Af = 10^{\left(\sum \frac{\left| \log \left(\frac{predicted}{observed} \right) \right|}{n} \right)} \tag{3.28}$$

To evaluate over-fitting of the models, the Akaike information criterion (AIC) and the Bayesian Schwarz criterion (BIC) values were calculated from the equations given by Carrillo et al. (2017) (Equation 3.29 and 3.30), respectively. Both parameters are related

to the performance of the models, however BIC value is more conservative with a stricter penalty. The smallest AIC and BIC values indicated the most accurate and parsimonious model (Quinn & Keough, 2002).

$$AIC = N\left[ln\left(\frac{2\,\pi\,\sigma^2}{N}\right) + 1\right] + 2 \tag{3.29}$$

$$BIC = N\left[ln\left(\frac{2\,\pi\,\sigma^2}{N}\right) + 1\right] + P.\ln(N) \tag{3.30}$$

where N is the number of observations; P is the number of parameters of the model and σ^2 is the variance calculated from the mean square error (MSE).

3.2. Results and Discussion

3.2.1. Characterization of the Freshly Squeezed Verjuice

Physicochemical (pH, titrable acidity, total soluble solid content) and optical properties (absorption coefficient, turbidity, CIE color parameters) of verjuice were summarized in Table 3.1. Physicochemical properties clearly shown that the verjuice used in this study was a highly acidic juice because of very low pH (2.63) and high titratable acidity content (2.89%). Total soluble solid or sugar content of verjuice was also very low (4.29 °Brix) in comparing with mature grape juice which is having minimum 15 °Brix content according to Codex Standart (Alimentarius, 1981b). The reason of low sugar content in verjuice is due to harvesting of grapes before maturation. Similarly, Hayoglu et al. (2009) used verjuice having pH of 2.91, titratable acidity of 3.00% and total soluble solid content of 4.5 °Brix. The slight differences between the compositions of verjuice samples in these studies were attributed to different soil, location and climate conditions (Bates et al., 2001; Somogyi et al., 1996).

In this study, turbidity value (37.94 NTU) and absorption coefficient (25.80 cm^{-1}) of verjuice exhibited cloudy and opaque structure in comparison to optical properties of commercial grape juice (turbidity: 0.87 NTU and absorption coefficient: 5.49 cm^{-1}) used in the study of Kaya and Unluturk (2016). CIE color parameters of verjuice were measured as L*: 29.20 (lightness), a*: 0.20 (redness) and b*: 3.07 (yellowness). These

results demonstrated that yellowness was also markedly detected in verjuice due to presence of carotenoid pigments that is responsible for yellow color in its composition (Lancaster, Lister, Reay, & Triggs, 1997).

Table 3.1. Physicochemical and optical properties of freshly squeezed verjuice

		Freshly squeezed verjuice	
pH		2.63	±0.02
TSS (°Brix)		4.29	±0.07
T.A. (%)		2.89	±0.01
Turbidity (NTU)		37.94	±2.01
Abs coef. (cm^{-1})		25.80	±0.05
Color	L*	29.20	±0.06
	a*	0.20	±0.00
	b*	3.07	±0.02

Results were presented as mean±standard error (n = 3). TSS: total soluble content.

3.2.2. The Effect of UV-C Irradiation (UV) on *S. cerevisiae* in Verjuice

The inactivation efficiency of UV-C irradiation on *S. cerevisiae* (NRRL Y-139) strains in the freshly squeezed verjuice was evaluated by circulating the juice eight times in UV system at 3.80 ml/s flow rate. Total UV exposure time (t_{uv}) and total processing time (t_{total}) for eight cycles of verjuice was calculated as 496 s and 912 s for this flow rate. During the process, the temperature increase in the juice due to heat generated by UV-C lamps was prevented by circulating water at 10 °C ($T_{water\ circulation}$) around the holding tank. The juice entered to the system at 16 °C (T_{juice}). The inactivation rate of the yeast cells per each cycle using different initial loads was depicted in Figure 3.8.

According to the Figure 3.8, the log reduction of *S. cerevisiae* after 8 cycles was 0.40±0.04 log CFU/mL and 0.54±0.02 log CFU/mL for high initial load and low initial load, respectively. The average juice temperature in the system was recorded as 18.1±2.2 °C during process. UV dose absorbed by verjuice was calculated from the actinometrical study as 178.37±0.16 mJ/mL (44.57±0.04 mJ/cm^2) at the end of UV process. Logarithmic reduction and actinometrical UV dose at each cycle during UV-C process was given in Table A.2 in Appendix A. The low initial microbial load resulted in slightly higher inactivation compared to the high initial load of *S. cerevisiae* in verjuice. Similarly, Kaya and Unluturk (2016) also reported a lower inactivation rate in white grape juice when it was inoculated with higher initial load of *S. cerevisiae*. The reason of this might be due to shadowing effect caused by high number of microorganisms preventing the UV light to reach each microorganism found in the medium (Karel & Lund, 2003). Gouma et al. (2015b) also studied inactivation of *S. cerevisiae* STCC1172 in apple juice subjected to UV-C irradiation. They found 0.51 log reduction of yeast cells at 3.92 J/mL UV dosage. Similarly, Keyser, Muller, Cilliers, Nel, and Gouws (2008) reported very low reduction of yeasts and moulds counts in orange juice, i.e. 0.30 log CFU/mL, after exposure of 1.38 J/mL UV dosage. UV dose in this study was lower than those of reported by Keyser et al. (2008). This was due to the higher absorption coefficient of verjuice (25.80±0.05 cm^{-1}) and higher depth of juice layer (5 mm). The reason of low efficacy of the UV-C irradiation in this study might be also attributed to presence of suspended particles and laminar flow regime of the juice passing through the UV system. Koutchma et al. (2004) stated that particles in turbulent flow system provides better mixing and they are longer exposed to the UV-C light resulting better inactivation. Besides, it was reported that microorganisms naturally growing in juice products were more resistant to UV-C irradiation (Unluturk & Atilgan, 2014). Additionally, low level of inactivation in this study might be due to the resistivity of the acid adapted yeast cells to UV-C light. It is also worth mentioned that the yeast cells have larger sizes and different DNA structure than bacteria. This may also affect the inactivation performance of UV-C light (Bintsis et al., 2000; Tran & Farid, 2004). Thus, hurdle strategies by using mild heating with the UV-C irradiation can be an alternative method to increase the efficiency of the UV-C system.

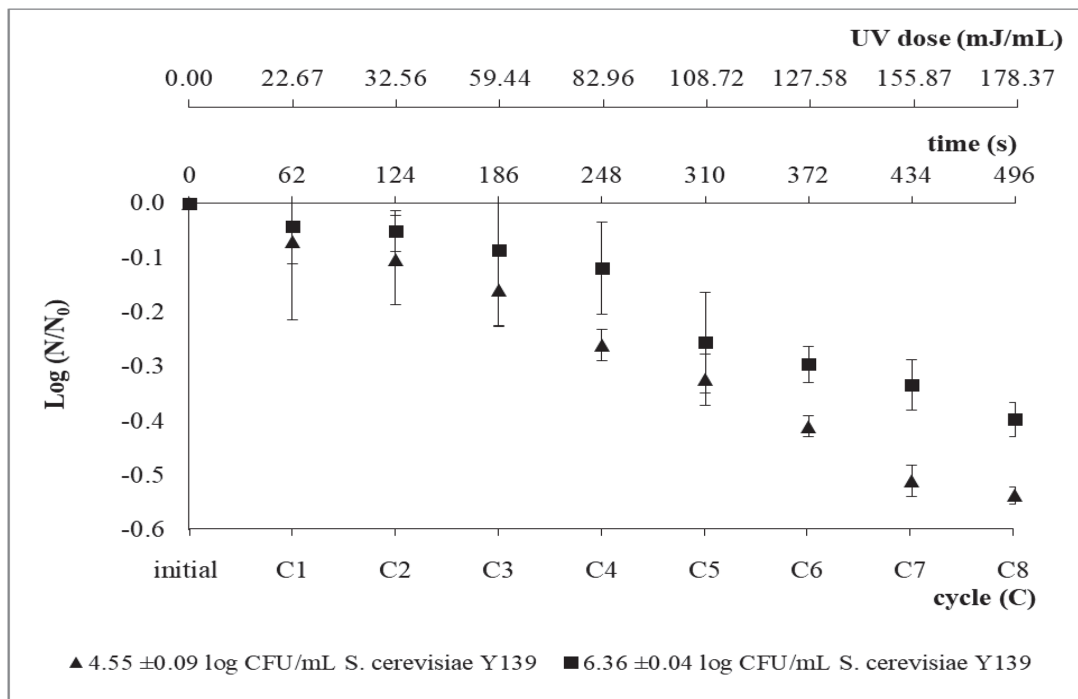


Figure 3.8. Inactivation of acid adapted *S. cerevisi*ae (NRRL Y-139) at low (▲) and high (■) initial load in freshly squeezed verjuice by using UV-C irradiation

3.2.3. The Effect of Mild Heating (MH) on *S. cerevisiae* in Verjuice

Two different mild heating processes were applied in the UV-C reactor system without turning on UV lamps. Logarithmic reductions of *S. cerevisiae* (NRRL Y-139) strains per each cycle were plotted in Figure 3.9 (a&b). The first mild heating process (MH1), i.e. 47.04±2.90°C, was resulted in 0.96±0.25 log reduction of yeast cells after the 8th cycle (Figure 3.9a). However, the second mild heating process (MH2), i.e. 50.08±3.25°C, was provided a higher inactivation, i.e., 3.13±0.05 log CFU/mL (Figure 3.9b). Logarithmic reduction and temperature of each cycle of the mild heating processes were given in Table A.3 in Appendix A. The reason of having more inactivation in MH2 could be due to the heating effect. Since average temperatures of the verjuice throughout eight cycles during MH1 and MH2 processes were recorded as 47.04 °C and 50.08 °C. Gouma et al. (2015b) reported that *S. cerevisiae* do not show significant decrease up to 52°C (2.7 min). The findings of this study were in agreement with their study. The maximum temperature of verjuice at the end of the 8th cycle during MH1 treatment was recorded as 49.10±0.52°C which resulted in an insignificant reduction (0.96±0.25 log CFU/mL) (see Table A.3i).

It was shown that mild heating processes (MH1 & MH2) were not enough to meet the required pasteurization conditions mandated by FDA (5 log reduction). For this reason, it was decided to combine mild heating process with the UV-C irradiation to increase efficacy of the UV system.

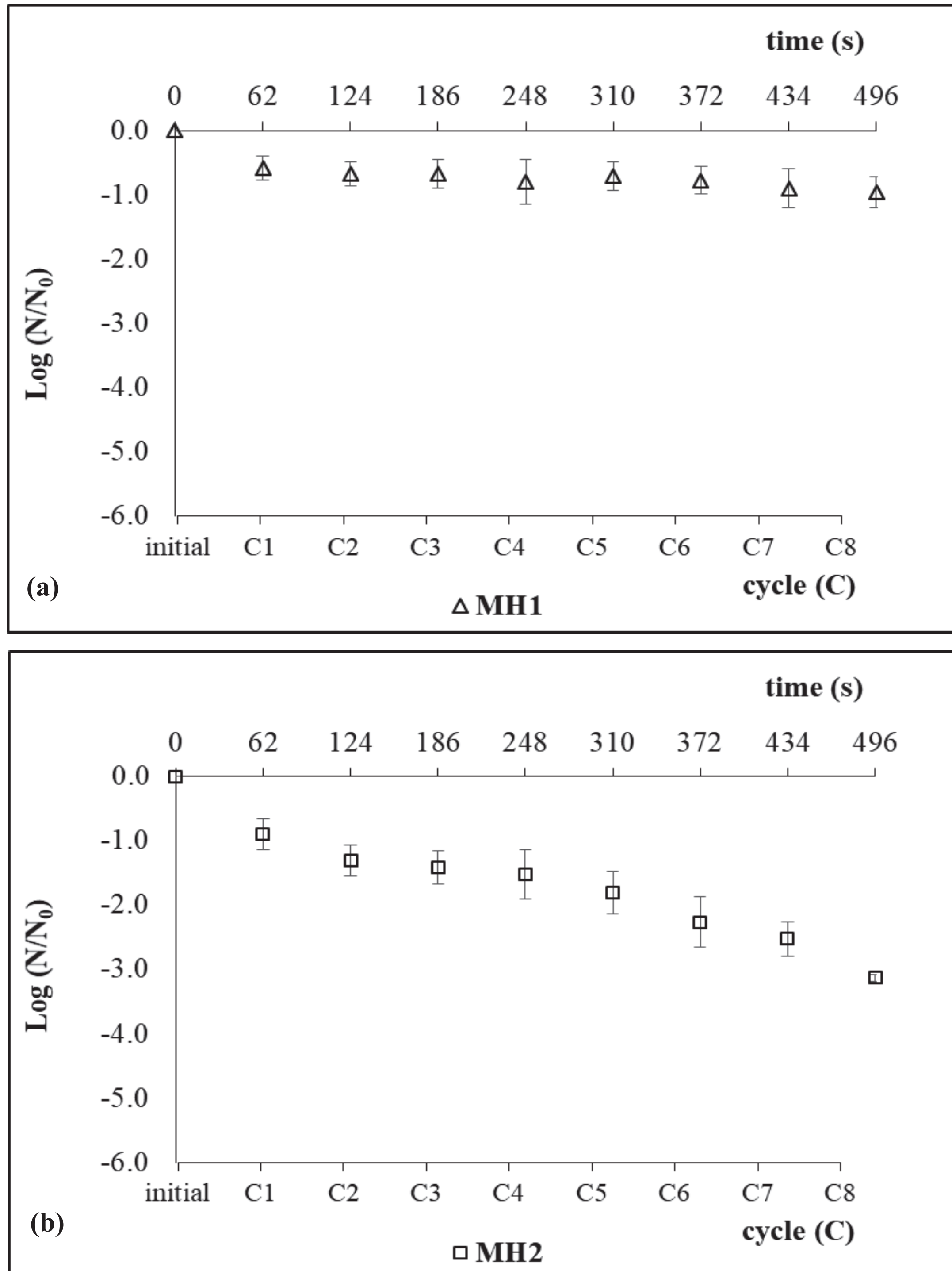


Figure 3.9. Inactivation of acid adapted *S. cerevisiae* (NRRL Y-139) cells in freshly squeezed verjuice by using mild heating (Average verjuice temperatures were a: (MH1) 47.04±2.90°C , b: (MH2) 50.08±3.25°C)

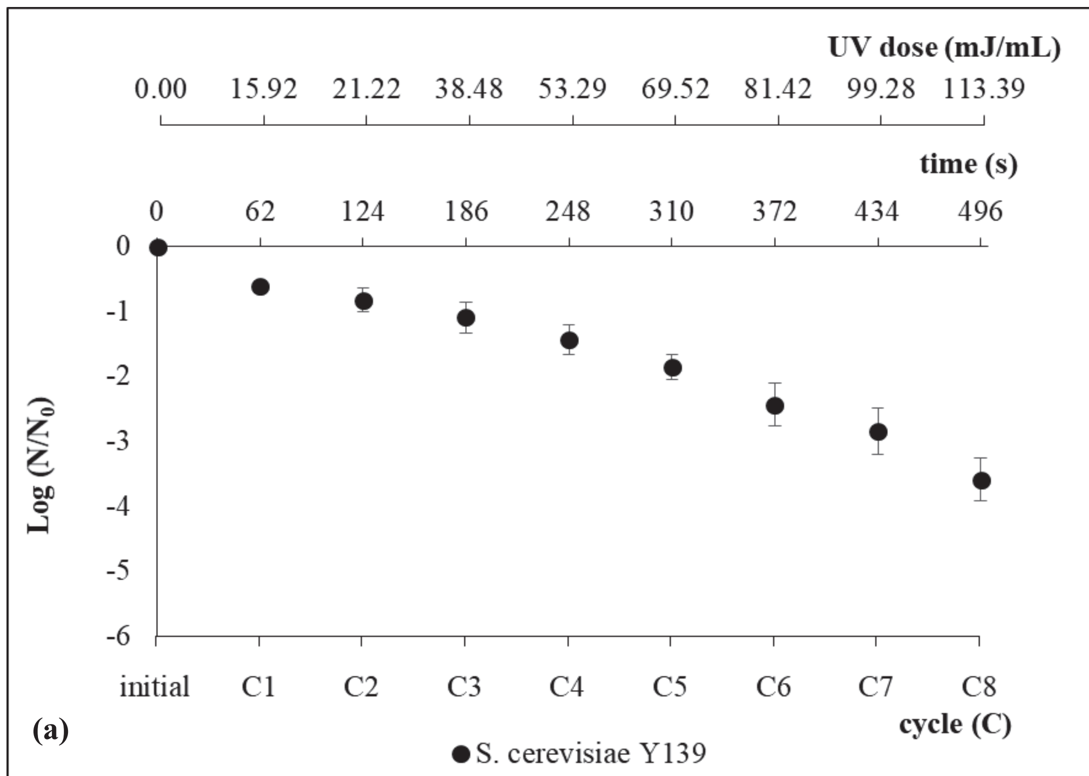
3.2.4. The Effect of Combined Treatment (UV+MH) on *S. cerevisiae* in Verjuice

UV-C irradiation and mild heating at the same process conditions was combined as a hurdle technology in order to achieve 5 log reductions in the number of target microorganism recommend by FDA. The log reduction of the *S. cerevisiae* (NRRL Y-139) in freshly squeezed verjuice per each cycle during UV-C irradiation combined with mild heating (hurdle) processes were shown in Figure 3.10 (a & b). Logarithmic reduction data of *S. cerevisiae* versus the actinometrical UV dose (J/mL) and temperature changes (°C) during each cycle in combined processes were listed in Appendix A. (Table A.4i and A.4ii).

In the combined process 1 (UV + MH1), average temperature of verjuice during eight cycles was measured as 48.22±2.17°C. After 8 cycles, 3.59±0.33 log CFU/mL reduction of *S. cerevisiae* was obtained by applying UV dose of 113.39±0.001 mJ/mL (28.33±0.00 mJ/cm^2) (Figure 3.10a).

In the second combined process (UV + MH2), *S. cerevisiae* cells were completely inactivated (5.16±0.24 log CFU/mL) after 496 s (at the end of 6 cycles) with the UV dose of 77.98±0.26 mJ/mL (19.49±0.07 mJ/cm^2) (Figure 3.10b). The average temperature of verjuice during eight cycles was measured as 51.24±2.74°C. Gouma et al. (2015b) found higher inactivation rate for yeasts by combining higher UV-C dose and mild heating at slightly higher temperature. They applied 2.90 J/mL UV dose at 55°C and obtained 1.3 log reduction of *S. cerevisiae.* However, >5 log reduction of *S. cerevisiae* was achieved by using the same UV dose at 60°C. Gayan et al. (2014) reported similar reduction levels for *E. coli* O157:H7 in apple juice. They claimed 1.44, 2.86 and 5.47 log reduction of *E. coli* after 20.33 J/mL at 55, 57 and 60°C, respectively. At the same temperatures, 2.57, 3.41 and >6 log reduction of *E. coli* was achieved in orange juice exposed to 13.55 J/mL of UV dose (Gayan et al., 2012).

The inactivation level of *S. cerevisiae* in freshly squeezed verjuice samples was higher than 5 log in the UV + MH2 treatment. This combined treatment, i.e. UV-C irradiation with MH2 ($T_{juice\ inlet}$: 50 °C and $T_{water\ circulation}$: 60 °C), was selected for pasteurization of the freshly verjuice in the further shelf life studies.



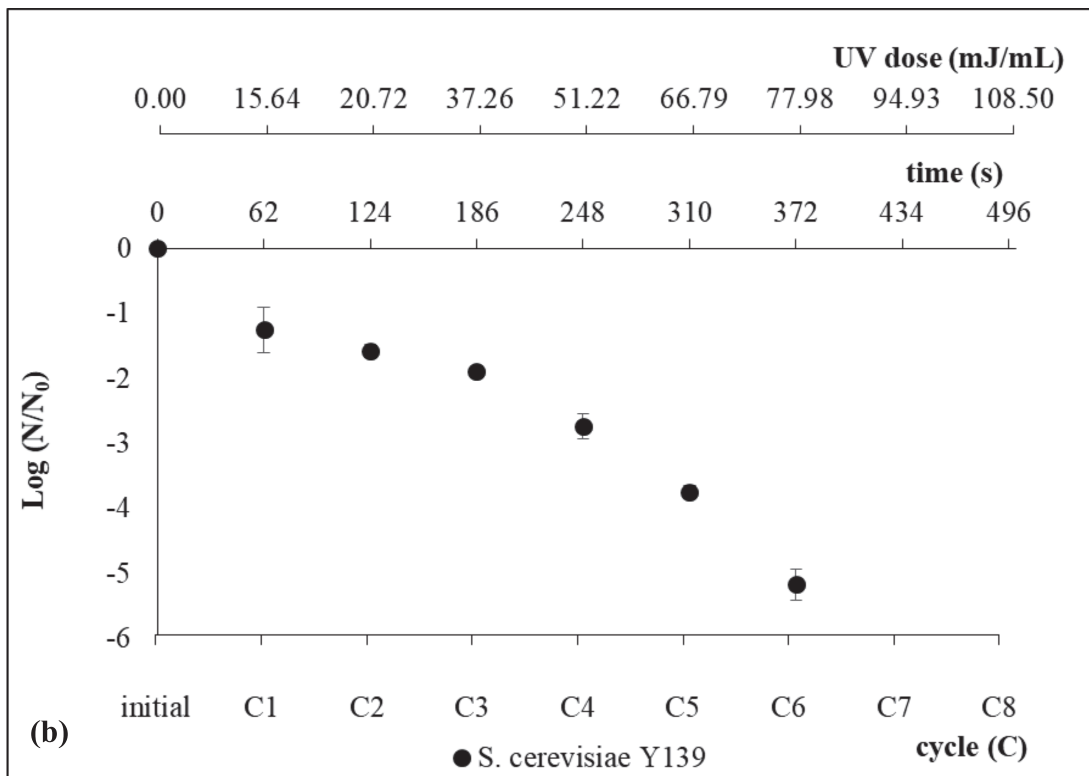


Figure 3.10. Inactivation of acid adapted *S. cerevisiae* (NRRL Y-139) cells in freshly squeezed verjuice by using UV-C irradiation combined with mild heating (Average temperatures of the verjuice were a: (UV+MH1) 48.22±2.17°C, b: (UV+MH2) 51.24±2.74°C)

3.2.5. Modelling and Kinetics of *S. cerevisiae* Inactivation in verjuice by UV, MH and UV+MH processes

Log-linear model by Van Boekel (2002), log-linear plus shoulder and log-linear plus tail model by Geeraerd, et al. (2000) and Weibull models by Mafart et al. (2002) were applied to logarithmic reduction data of *S. cerevisiae* (NRRL Y-139) inoculated into freshly squeezed verjuice subjected to UV-C, mild heating and UV-C combined with mild heating processes. In order to compare and determine the best fitting model for each processes, kinetic parameters of the models, RMSE, B_f, A_f, AIC and BIC values were listed in Table 3.2. B_f and A_f values were the model prediction parameters commonly used in literature (Gayan et al., 2016; Gouma et al., 2015b; Singh, Singh, Singh, Nayak, & Ghanshyam, 2017). According to table, Weibull model was the best model predicted the data with the smallest RMSE, the B_f value and the A_f values closest to 1. The inactivation kinetics of *S. cerevisiae* were best described by Weibull model with a B_f value of 1.00, i.e. the predicted log reductions of *S. cerevisiae* were in perfect agreement with the observed data. Bf value for inactivation data of S. cerevisiae obtained from UV+MH2 treatment was calculated as 1.08. Therefore, it was an acceptable model according to the classification of Bf given in Amazquita et al. (2011) and Ross (1999). Besides, the inactivation kinetics of *S. cerevisiae* were best identified by Weibull model with the lowest A_f values, i.e. the predicted log reductions were closest to the observed values. Similarly, Weibull model was the best model fitted to inactivation data of *S. cerevisiae* in verjuice with the smallest AIC values (Table 3.2). BIC values of UV (high), MH1, MH2 and UV+MH1 treatments were the lowest for Weibull model. BIC values calculated for the linear and Weibull models fitting the data obtained in UV (low) and UV+MH2 treatments were not very different from each other (differences were less than 2). Dziak, Coffman, Lanza, and Li (2012) quoted that “the size of a difference in AIC or BIC between models is practically significant. For example, an AIC difference between two models of less than 2 provides little evidence for one over the other”. Therefore, it was decided that the inactivation kinetics of *S. cerevisiae* were best described by Weibull model with the smallest RMSE, B_f, A_f and AIC values.

Table 3.2. Goodness-of-fit (RMSE, Bf and Af) and model overfitting (AIC, BIC) parameters for the log-linear, log linear plus shoulder, log linear plus tail and Weibull models

		RMSE	B_f	A_f	AIC	BIC
UV (high)	**Log-linear**	0.03	1.00	1.01	-53.34	-53.14
	Log-linear + shoulder	0.04	1.00	1.01	-51.95	-49.56
	Log-linear + tail			NS		
	Weibull	0.03	1.00	1.00	-56.64	-54.24
UV (low)	**Log-linear**	0.02	1.00	1.01	-60.4	-60.2
	Log-linear + shoulder	0.02	1.00	1.01	-59.02	-56.62
	Log-linear + tail			NS		
	Weibull	0.02	1.00	1.00	-62.36	-59.96
MH1	**Log-linear**	0.17	1.00	1.03	-24.15	-23.95
	Log-linear + shoulder			NS		
	Log-linear + tail	0.10	1.00	1.02	-32.82	-30.43
	Weibull	0.04	1.00	1.01	-51.95	-49.55
UV+MH1	**Log-linear**	0.18	1.01	1.05	-22.94	-22.74
	Log-linear + shoulder	0.19	1.01	1.05	-21.9	-19.5
	Log-linear + tail			NS		
	Weibull	0.15	1.00	1.04	-26.74	-24.35
MH2	**Log-linear**	0.23	1.00	1.06	-18.69	-18.49
	Log-linear + shoulder			NS		
	Log-linear + tail			NS		
	Weibull	0.21	1.00	1.06	-21.2	-18.81
UV+MH2	**Log-linear**	0.36	1.12	1.23	-6.04	-6.09
	Log-linear + shoulder	0.40	1.12	1.23	-4.7	-2.8
	Log-linear + tail			NS		
	Weibull	0.36	1.08	1.17	-6.04	-4.15

RMSE: Root mean squared error. B_f: Bias factor. A_f: Accuracy factor. AIC: Akaike information criterion. BIC: Bayesian Schwarz criterion. NS: not suitable model.

Weibull model parameters, i.e. delta (δ) and p obtained from GlnaFiT software, were used to calculate time required for pasteurization of freshly squeezed verjuice based on 5D reduction of *S. cerevisiae* (NRRL Y-139) (Table 3.3). It was predicted that verjuice having high (6.36±0.04 log CFU/mL) and low (4.55±0.09 log CFU/mL) initial load must be exposed to UV-C irradiation for at least 49.63 and 54.73 min, respectively in order to achieve 5 log reductions of *S. cerevisiae* (Table 3.3). But it was shown that UV-C

treatment for 8.96 min used in this study was insufficient for pasteurization of verjuice because the maximum log reduction was achieved as 0.54±0.02 log CFU/mL (Figure 3.8). 5D values for MH1, MH2 and UV+MH1 treatments were predicted as 24.11, 18.54 and 11.40 min using Weibull model. The only process resulted in complete inactivation of *S. cerevisiae* resulting in full pasteurization of verjuice was the hurdle process 2 (UV+MH2). 5D value of this process was estimated as 6.82 min using Weibull model.

The UV-C inactivation curve of *S. cerevisiae* in verjuice inoculated at high and low initial loads revealed downward concavity since p values (p_{UV}) were calculated as 1.40 and 1.16 (p>1), respectively (Table 3.3). UV-C combined with mild heating treatments (p_{UV+MH1}: 1.32 and p_{UV+MH2}: 1.28) also showed downward concavity. The reason of this phenomenon can be due to the non-lethal UV-dose for the resistant yeast cells at the initial period of the process. After that, yeasts cells could be inactivated by the effect of heat. Similarly, Van Uden, Abranches, and Cabeça-Silva (1968) estimated p (or β) value as 1.5 for *S. cerevisiae* in a buffer solution having pH 3.5 after 50°C mild heat treatment. In the second mild heat treatment (MH2) (50.08±3.25°C), microorganisms tended to be killed immediately (upward concavity, i.e.p_{MH2}: 0.75). However, the first mild heat treatment (MH1) was not lethal (47.04±2.90°C) to microorganisms exhibiting a downward concavity in the inactivation data (p_{MH1}: 2.5). Van Boekel (2002) also reported that shape parameter (p or β) of the Weibull model decreases by increasing temperature reaching over the lethal temperatures (≥50 °C). Above the lethal temperature, microorganisms have become more sensitive to heat and cell damage has increased.

5D values calculated from the Weibull model (Table 3.3) can be compared with the one obtained in the actual experiments. The complete inactivation of the cells (5.16±0.24 log CFU/mL) was achieved within 6.2 min by UV+MH2 treatment in the actual experiment (Figure 3.10b) while 5D value of this process was calculated as 6.82 min from the Weibull model (Table 3.3). It could be said that the inactivation kinetics of the *S. cerevisiae* (NRRL Y-139) could be well explained by Weibull model. Weibull model was also used to explain the inactivation kinetics of the microorganisms in fruit juices exposed to UV-C irradiation. Unluturk and Atilgan (2014) found that the inactivation of *E. coli* K12 and lactic acid bacteria in white grape juice by UV-C irradiation was best described by Weibull model with the least RMSE values (0.001 and 0.0004). Baysal, Molva, and Unluturk, (2013) studied the inactivation kinetics of *A. acidoterrestiris* in white grape juice exposed to 0.38 mW/cm^2 UV-C dose. They found that Weibull model is suitable for inactivation data with low RMSE (0.213).

Table 3.3. Pasteurization time (5D values) for inactivation of *S. cerevisiae* (NRRL Y-139) in freshly squeezed verjuice calculated by means of Weibull Model and synergism of processes

	delta (δ)	p	Log N_0	RMSE	R^2	D (s)	D (min)	Exp. 5D (s)	Exp. 5D (min)	Theo. $5D_{UV+MH}$ (min)	Synergism (%)
UV (high load)	940.64	1.40	6.36	0.028	0.9725	940.6	15.68	2977.8	49.63		
UV (low load)	819.53	1.16	4.55	0.020	0.9918	819.5	13.66	3284.1	54.73		
MH 1 (50&55C)	760.08	2.50	3.89	0.190	0.9509	760.2	12.67	1446.6	24.11		
UV+MH 1 (50&55 °C)	201.42	1.32	4.59	0.147	0.9878	201.4	3.36	684.0	11.40	16.74	31.90
MH 2 (50&60 °C)	129.14	0.75	4.46	0.220	0.9576	129.1	2.15	1112.6	18.54		
UV+MH 2 (50&60 °C)	116.54	1.28	4.84	0.361	0.9682	116.5	1.94	408.9	6.82	13.85	50.79

3.2.6. Synergistic lethal effect of Combined UV+MH Treatment

The 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 & Lund, 2003). On the other hand, microorganisms exposed to heat treatment can be inactivated by oxidation, protein denaturation and membrane damage (Smelt & Brul, 2014). In addition to these individual inactivation mechanisms, synergistic lethality of the combined UV-C and mild heating could be also explained by the effect of heat inhibiting the DNA repair system of the cell which was previously damaged by exposure to UV-C irradiation. Initially, DNA could repair itself on the non-lethal dose of UV-C and low temperature (photoreactivation). However, further application of heat may cause irreversible damage on the DNA of the cell (Gayan et al., 2012).

Results of combined processes indicate that there was a synergism between UV-C irradiation and mild heating when they were applied simultaneously rather than individually. Pasteurization times (5D) for inactivation of *S. cerevisiae* (NRRL Y-139) in verjuice subjected to UV-C, mild heating and combined processes were shown in Figure 3.11. The exposure times for individual UV-C treatment and mild heating treatments (MH1, MH2) to achieve 5-log reduction of *S. cerevisiae* was predicted as 54.73 min, 24.11 min and 18.54 min, respectively. In the first hurdle process (UV+MH1), the required exposure time for 5D process of verjuice was found as 11.4 min while in the second hurdle process (UV+MH2) this value was estimated as 6.82 min. Thus, it was revealed that exposure times required for 5D processes of verjuice, when the processes were combined, were much shorter than processes applied separately. Synergistic effect of the UV-C combined with mild heating processes were depicted in Table 3.3. In the first hurdle process (UV+MH1), 31.90% synergism was calculated when the verjuice was treated with 113.39±0.001 mJ/mL (28.33±0.00 mJ/cm^2) UV dose at 48.22±2.17°C. This means that the cells were inactivated 31.90% times more in the process when UV-C and mild heating processes were combined. Furthermore, the synergistic lethal effect was calculated as 50.79% in the second combination (UV+MH2), i.e. 77.98±0.26 mJ/mL (19.49±0.07 mJ/cm^2) UV dose at 51.24±2.74°C. The reason of higher synergistic effect could be attributed to the dominant lethal effect of heat in the UV+MH2 (51.24±2.74°C). Gouma et al. (2015b) observed that the lethal effect of UV light was significantly improved by heating at or above 50°C. They found maximum 33% synergism in the

combination of UV and mild heating at 55°C. Gayan et al. (2014) obtained similar synergism (50%) on the inactivation of *S. aureus* in apple juice by combining UV-C (20.3 J/mL) and mild heating at 52.5°C. It was also reported by several studies that synergism was affected by an increase of temperature reaching the highest value at 55°C (Gayan et al., 2012; Gouma et al., 2015b).

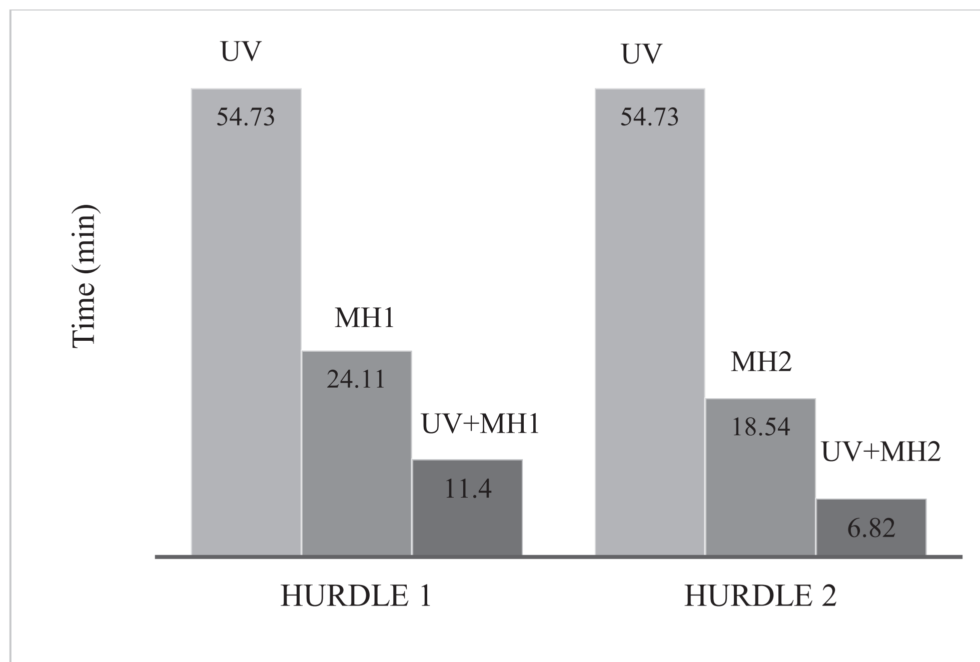


Figure 3.11. The required pasteurization time (5D) for inactivation of *S.cerevisiae* (NRRL Y-139) in verjuice subjected to UV-C (UV), mild heating (MH1 & MH2) and combined processes (UV+MH1 & UV+MH2)

In conclusion, the second hurdle process (UV+MH2) conducted at 77.98±0.26 mJ/mL (19.49±0.07 mJ/cm^2) UV dose and 51.24±2.74°C was selected as the pasteurization process conditions for verjuice and was used for shelf life studies.

3.3. Conclusions

In this chapter, suitable pasteurization conditions for verjuice providing 5-log reduction of target microorganism, i.e. *S. cerevisiae* (NRRL Y-139), using UV-C irradiation, mild heating and the combination of UV-C and mild heating processes were determined. Yeast cells were initially adapted to acidic conditions and verjuice having two different initial loads of acid adapted *S. cerevisiae* were exposed to UV-C irradiation in a continuous flow UV system. Mild heating and combined treatments were applied by

circulating hot water at two different mild temperatures in the same UV-C system. Inactivation behavior of acid adapted *S. cerevisiae* in treated verjuice was evaluated by using log linear and several non-log linear kinetic models.

UV-C treatment alone was not sufficient for pasteurization of verjuice. Maximum logarithmic reduction of *S. cerevisiae* was found as 0.54±0.02 log CFU/mL (178.37±0.16 mJ/mL dose) after 8.96 min exposure to UV-C light in the system at 18.1±2.2 °C. Verjuice was also not completely pasteurized by mild heat treatments e, i.e. maximum 3.13±0.05 log CFU/mL reductions were obtained after 8.96 min exposure at 50.08±3.25°C (MH2). However, complete pasteurization of verjuice was achieved by a combined treatment (UV+MH2), i.e. 5.16±0.24 log CFU/mL reduction of *S. cerevisiae,* applied at the UV dose of 77.98±0.26 mJ/mL and 51.24±2.74°C for 6.2 min.

Weibull was the best fitted model to obtain kinetic inactivation parameters of acid adapted *S. cerevisiae* in verjuice treated with UV-C, mild heating and combined treatments. The complete inactivation of the cells (5.16±0.24 log CFU/mL) was achieved within 6.2 min by UV+MH2 treatment in the actual experiment while the time for 5-log reduction (5D value) of this process was calculated as 6.82 min using the Weibull model. The synergistic lethal effect of the UV-C irradiation and mild heat treatment on *S. cerevisiae* in verjuice was observed. Synergism was increased reaching up to maximum 50.79% when UV-C irradiation was combined with mild heating. It was concluded that UV-C irradiation at mild heat temperature greater than 50 °C can ensure the pasteurization of verjuice.

CHAPTER 4

SHELF LIFE OF VERJUICE PASTEURIZED BY A COMBINED UVC-MILD HEAT TREATMENT

In recent years, consumer demands have been increased to more fresh-like and healthy fruit juices with a high sensorial and nutritious quality. Thermal pasteurization at high temperatures is the most convenient method and commonly used for pasteurization of fruit juices. Although the thermal process provides an effective pasteurization, long and stable shelf life for the fruit juice, it causes many quality problems such as permanent loss of flavour and taste, degradation of nutrients and undesirable browning reactions emerged from heating (Khan et al., 2017; Tiwari, O'Donnell, Muthukumarappan, & Cullen, 2009b; Walkling-Ribeiro et al., 2008; Wang Guo, Ma, Zhao, & Zhang, 2018). Thus, several nonthermal processing technologies such as High Hydrostatic Pressure, Pulse Electric Field, UV-C radiation, Ultrasound etc. have been emerged and developed as an alternative to thermal process in order to produce fruit juices with a minimum quality loss (Dunne & Kluter, 2001; Jimenez-Sanchez, Lozano-Sanchez, Segura-Carretero, & Fernandez-Gutierrez, 2017; Raso & Barbosa-Canovas, 2003). As one of the nonthermal technologies, UV-C irradiation at 254 nm has a germicidal effect on microorganisms by damaging of their DNA (Koutchma et al., 2009). Thus, UV-C irradiation has been commonly used for inactivation of pathogenic and spoilage microorganisms in foods including disinfection of water and air (Pereira & Vicente, 2010), surface disinfection of the foods (Turtoi & Borda, 2014) and disinfection of liquid foods and beverages (Begum et al., 2009; Koutchma et al., 2004). Recent studies showed that UV-C irradiation can be successfully used for decontamination of fruit juices such as apple juice (Guerrero-Beltran & Barbosa-Canovas, 2004; Walkling-Ribeiro et al., 2008), orange juice (Pala & Toklucu, 2013b), pomegranate juice (Pala & Toklucu, 2011), pineapple juice (Chia, Rosnah, Noranizan, & Wan Ramli, 2012), tropical juices (Keyser et al., 2008), lemon melon juice blend (Kaya et al., 2015) and grape juice (Kaya & Unluturk, 2016; Unluturk & Atilgan, 2014). Additionally, some of the literature reported that inactivation efficacy of UV-C irradiation can be improved when combined with mild

heating (Carrillo et al., 2017; Gayan et al., 2013; Gayan et al., 2016; Gouma et al., 2015a; Gouma, et al., 2005b)

The objective of this chapter was to evaluate several quality characteristics of verjuice pasteurized by a UV-C irradiation combined with the mild heating process (the optimum process conditions were determined and given in the chapter 3) during twelve weeks storage period at refrigerated conditions (4 °C). Firstly, microbial quality of the processed verjuice was monitored by following its total aerobic mesophilic, coliforms, yeasts and molds counts. Besides, physicochemical and optical properties such as pH, total soluble solids content, titratable acidity, absorption coefficient, turbidity and color parameters, i.e. L* (lightness-darkness), a*(redness-greenness), b* (yellowness-blueness), hue angle (h°), chroma (C*), total color difference and browning index of verjuice were analyzed during 12 weeks of storage. Untreated freshly squeezed verjuice and thermally pasteurized verjuice were used as negative and positive controls, to make a comparison.

4.1.Materials and Methods

4.1.1. Conventional Thermal Pasteurization (P)

4.1.1.1. Determination of Thermal Inactivation Kinetics Parameters of *S. cerevisiae* in Verjuice

Thermal inactivation kinetics of microorganisms in foods are explained by the first-order linear kinetic model developed by Bigelow, Ball, and Stumbo based on calculation of D and z values (Stumbo, 1973). According to this model, distribution of the heat is uniform in the foods during processing thus thermal inactivation kinetics is linear (Chen, Campanella, Corvalan, & Haley, 2008). This equation was given in chapter 3 (Equation 3.19). Decimal reduction time (D) value in Equation 3.19 was determined as the time needed for 90% inactivation of the cells at a specific temperature (Silva & Gibbs, 2012; Van Boekel, 2002). The practical calculation of D value could be also obtained from Equation 4.1 (Ray, 2004).

$$D_T = -\frac{t}{\log N - \log N_0} \quad (4.1)$$

The equation 4.1 relates changes of logarithmic reduction as a function of time at certain temperature. The relationship between the logarithm of D values and temperature was expressed as z value of microorganisms. z value was explained as the temperature increase or decrease required to reduce D value by one logarithm (Silva & Gibbs, 2012; Van Boekel, 2002) and calculated from the Equation 4.2:

$$z = \frac{T_2 - T_1}{\log D_1 - logD_2} \quad (4.2)$$

where D_1 and D_2 are D values at the two different temperatures T_1 and T_2, respectively. Thermal inactivation kinetic parameters (D and z values) of the target microorganism in verjuice were determined by constructing a thermal death time (TDT) curve developed by Bigelow (1921) shown in Figure 4.1 (a&b). Firstly, number of logarithm reduction of microorganisms (log CFU/mL) plotted against time. D value for each temperature was calculated as the inverse of the slope obtained from the linear regression equation of the curve (D: -1/slope in Figure 4.1a). Then, z value was calculated as the inverse of the slope (z: -1/slope in Figure 4.1b) obtained from the logarithm of D value plotted versus temperature.

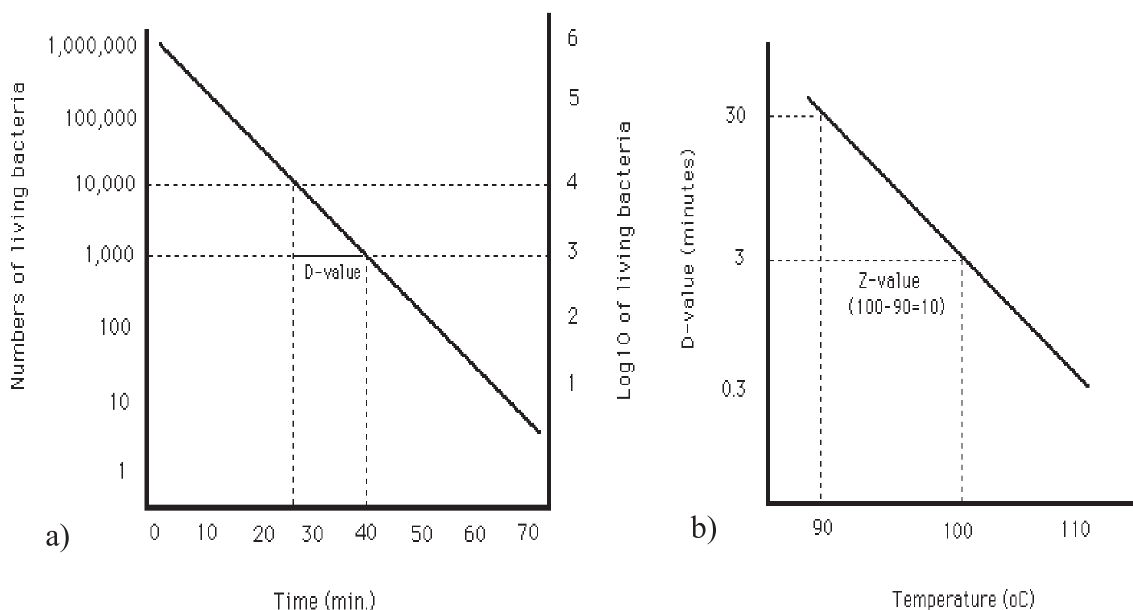


Figure 4.1. Thermal Death Time (TDT) curves for calculation of D (a) and z (b) values

D and z values were used to determine the thermal pasteurization condition for verjuice. The aim was to achieve 5-log reduction in the number of target microorganism in verjuice (U.S.FDA, 2000). For this purpose, *S. cerevisiae* (NRRL Y-139) strains were firstly adapted to acidic conditions of verjuice (pH 2.7) as explained in the section 3.1.4.2. Then, freshly squeezed verjuice (2.7 mL) was placed into the glass tubes (diameter less than 1 cm) and transferred into the water bath set at desired temperatures changing from 50 °C to 80 °C. Once verjuice tubes were reached to a desired temperature, 300 µL of acid adapted *S. cerevisiae* culture (10^7 CFU/mL) was inoculated and different heating times changing from 2 s up to 960 s were applied. After several treatments of temperature-time combinations shown in Table 4.1, *S. cerevisiae* cells in verjuice were counted on Potato Dextrose Agar (PDA) by surface plating method. Survival numbers of *S. cerevisiae* (log CFU/mL) were plotted as a function of time (s). D values of acid adapted *S. cerevisiae* (NRRL Y-139) in freshly squeezed verjuice at selected temperatures (50, 55, 60, 65, 70, 75, 80 °C) were obtained from the slope of the graph (Figure A.3) and listed in Appendix A (Table A.5). Gabriel (2012) reported D value for *S. cerevisiae* (BFE-39) in the apple juice heated to 55°C as 6.10 min (366 s). This value is higher than our findings ($D_{55°C}$: 78.33 s). The differences of D value are might be due to various factors, i.e. differences in *S. cerevisiae* strains, type of juice, amount of juice aliquots used in glass test tubes, type of the equipment and inactivation model applied to predict D value. Gabriel (2012) used the dynamic model developed by Baranyi and Roberts (1994) to characterize the growth/death kinetic parameters of *S. cerevisiae.* D value of *S. cerevisiae* (BFE-39) was calculated by obtaining the negative reciprocal of the death rate (k_d) of the inoculated microorganisms in heated apple juice. In comparison, D value in this study was estimated from the log-linear model. D value of *S. cerevisiae* (NRRL Y-139) was reported to be higher than bacteria in fruit juices (Gouma et al., 2015b; Tran & Farid, 2004).

The z value of acid adapted *S. cerevisiae* was estimated as 11.50±0.05 °C from the slope of Thermal Death Time curve (z: 1/slope) plotted with logarithmic D values versus corresponding temperatures (Figure A.4 given in Appendix A). Gouma et al. (2015b) determined z value of *S. cerevisiae* STCC 1172 as 3.75°C in apple juice. The reason of obtaining higher z value in this study could be a result of using acid adapted cells in verjuice. Besides, z value of acid adapted *S. cerevisiae* NRRL (Y-139) were higher than the several acid adapted bacteria species in fruit juices (Mazzotta, 2001).

Typical pasteurization conditions for fruit juices are 65 °C for 30 min, 77 °C for 1 min, or 88 °C for 15 s (Miller & Silva, 2012). Recently, fruit juices have begun to be processed at mild heat temperatures between 70 and 72 °C in order to protect the juice quality (Timmermans et al., 2011). FDA has also recommended a minimum temperature (71.1 °C) and time (3 s) combinations for pasteurization of high acidic food products (U.S. FDA, 2004). Mild Temperature Short Time (MTST) heat processing which have a limited effect on product characteristics uses temperatures < 80 °C and holding times 30 s (Petruzzi et al., 2017). The results of this study showed that 72 °C and 18 s was adequate for pasteurization of verjuice. In other words, > 5 log CFU/mL reduction of *S. cerevisiae* (NRRL Y-139) was achieved in verjuice at these processing conditions. Thus, 72 °C and 18 s were chosen as thermal pasteurization conditions in this study.

Table 4.1. Different temperature and time combinations for D and z values calculations

Temperature (°C) **Time (s)**	50 °C	55 °C	60 °C	65 °C	70°C	75 °C	80 °C
2							X
3							X
5						X	X
8					X		
10						X	X
15						X	
16					X		
20						X	
30				X	X		
60			X	X	X		
90			X	X			
120	X	X		X			
180			X				
240	X	X					
360	X	X	X				
480	X	X					
960	X	X					

4.1.1.2. Thermal Pasteurization of Verjuice

Thermally pasteurized verjuice (72 °C/18 s) was used as positive control in the shelf life study. Freshly squeezed verjuice were processed using the continuous flow thermal pasteurization system (Figure 4.2). Five hundred mililiter of verjuice was initially heated up to 70 °C on the hot plate by stirring in a beaker and transferred to the sample

tank of the heat pasteurization system (1). Then, verjuice in the sample tank was immediately pumped by a peristaltic pump (2) to the helical stainless steel tube of the system. Temperature of the helical tube was adjusted to 72 ° C by using a water bath (3). Verjuice was passed throughout the helical region of the system with a flow rate of 3.6 mL/s for 18 s, i.e., the pump frequency was adjusted to 65 rpm, and then cooled immediately within an ice bath (4) before analyses and storage. After the thermal process, continuous heat pasteurization system was disinfected by passing hot water several times throughout the system.

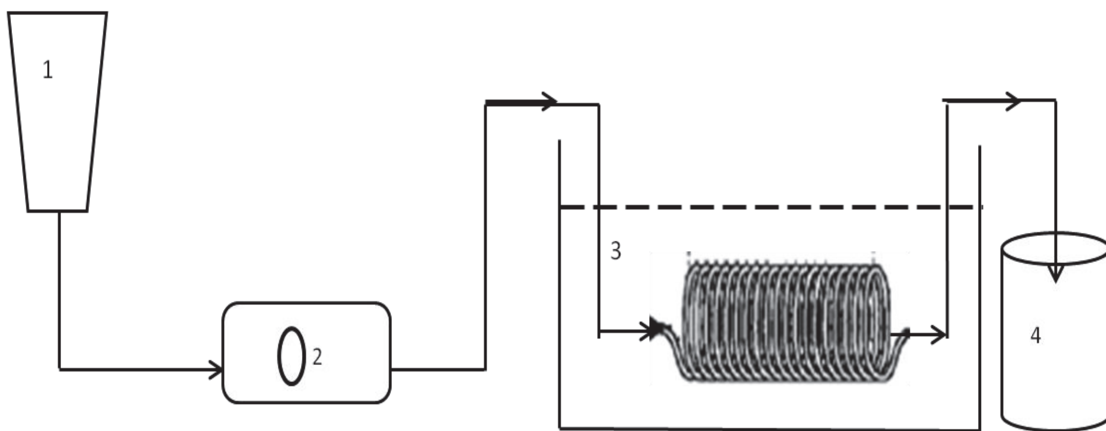


Figure 4.2. Continuous Heat Pasteurization System
(1: sample tank; 2: peristaltic pump; 3: heating coil; 4: cooler)

4.1.2. Pasteurization with Combined UV-C -Mild Heat Treatment (UV+MH2)

Freshly squeezed verjuice was prepared from frozen unripe grapes (stored in the freezer (-20 °C) using the preparation procedures outlined in section 3.1.2. Verjuice was pasteurized by using a combined UV-C irradiation and mild heat treatment (UV+MH2). UV-C irradiation (i.e., four UV lamps on configuration, six cycles, 3.80 mL/s flow rate, 6.2 min exposure time, 77.98±0.26 mJ/mL) and heat treatment (MH2 at 51.24±2.74°C) were simultaneously applied to achieve 5D microbial reduction in verjuice. These processing conditions were selected according to the results depicted in Chapter 3. For this purpose, 400 mL of freshly squeezed verjuice was pre-heated to 50 °C (T_{juice}) in a beaker and immediately placed in the sample tank of the continuous flow UV reactor system (section 3.1.8). Glass sample tank with jacket was equipped with a water bath

(Haake DL30, Thermo Electron Corp., Karlsruhe, Germany). The water temperature was adjusted to 60 °C ($T_{water\ circulation}$) and circulated around the sample tank to provide mild temperature condition in verjuice. Verjuice was also circulated six times throughout the combined UV-C-mild heating system with a flow rate of 3.80 ml/s (70 rpm pump frequency). The cycling time of verjuice was determined according to the inactivation study of combined UV+MH treatment in chapter 3 (section 3.2.4). Lamp configuration I (four lamps on) shown in Figure 3.7 was used for pasteurization of verjuice. The temperature of the verjuice within the sample tank and at the outlet of the UV-C system was checked by a K-type thermocouple (CEMDT-8891E, Shenzhen, China) during processes. Total UV exposure time (t_{uv}) and total processing time (t_{total}) for one cycle of 400 mL verjuice were previously recorded as 62 s and 144 s for a flow rate of 3.80 ml/s. Thus, these values were calculated as 372 s (t_{uv}) and 874 s (t_{total}) for six cycling times in the system. At the end of the six cycles, total UV dose absorbed by verjuice was measured as 77.98±0.26 mJ/mL (19.49±0.07 mJ/cm^2) based on the actinometric method explained in section 3.1.11 (chapter 3). The overall temperature of verjuice during pasteurization process was measured as 51.24±2.74°C.

4.1.3. Storage of Verjuice

Verjuice subjected to thermal pasteurization (P) at 72 °C for 18 s (positive control sample) and UV-C light assisted by mild heat (UV+MH2) at UV dose of 77.98±0.26 mJ/mL and 51.24±2.74°C for 6.2 min, and untreated freshly squeezed verjuice (U, negative control sample) were stored in sterile dark glass bottles (100 mL) at refrigerated conditions (4.22±1.15 °C) for 12 weeks. Microbiological and physicochemical quality of juice samples were monitored through the storage period (Figure 4.3). Processed samples stored at 4.2 °C were analysed on 0, 2, 4, 6, 8, 10, 12th weeks stored at 4.22 ºC. On the other hand, control samples were analysed weekly to determine microbiological and physicochemical quality of verjuice through storage period, i.e., 9 weeks.

4.1.4. Microbiological Analyses

Immediately after the opening of the bottles, each juice sample was submitted to sampling for microbiological analyses. Appropriate dilutions were made with 0.1%

peptone water. For this purpose total mesophilic aerobic counts (TMAC), yeasts and moulds (YM), and total coliforms were counted by spread plating on the Plate Count Agar (PCA, Merck, Darmstadt, Germany), the Potato Dextrose Agar (PDA, Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) and Violet Red Bile Agar (VRBA, Merck, Darmstadt, Germany) plates, respectively. PCA, PDA and VRBA plates were then incubated at 30 °C for 48 h for TMAC, at 25 °C for 2-5 days for YM counts and at 37 °C for 24 h for total coliforms, respectively. Results were expressed as Colony Forming Units per milliliter (CFU/mL).

4.1.5. Physicochemical Analyses and Optical Properties of Verjuice

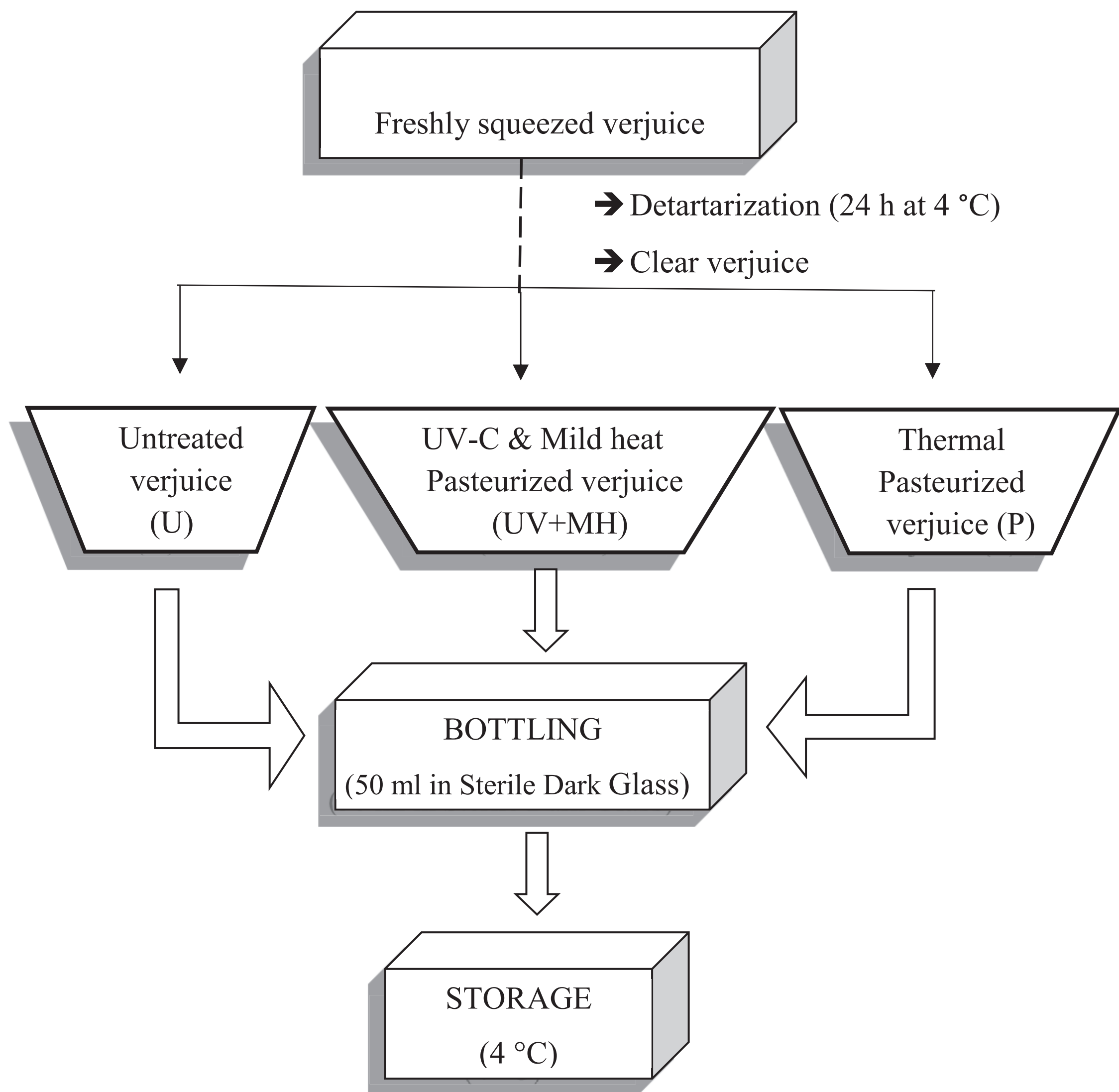
Physicochemical properties, i.e. pH, total soluble solid content (°Brix), titratable acidity (%), and optical properties, i.e. absorption coefficient (cm^1), turbidity (NTU), color (L*, a*, b*, C*, h°, ΔE, BI) of processed and untreated verjuice samples were determined during storage period.

4.1.5.1. pH

A benchtop pH meter (Inolab 7310, WTW series, Germany) was used to determine pH values of the verjuice. Before measuring the pH of verjuice, the pH meter probe was calibrated with standard solutions of pH 7.0 and pH 4.0, respectively. Then, the probe was immersed in 50 mL verjuice sample and pH value was recorded. All measurements were carried out at room temperature (20 °C).

4.1.5.2. Total Soluble Solid Content

The total soluble solid content (TSS) of verjuice was measured by means of a hand-held refractometer (Pocket refractometer PAL-1, Atago, Co, Ltd, Tokyo Tech., Japan) at room temperature (20 °C). Firstly, the apparatus was calibrated with distilled water. Then, the percentage of total soluble solid content of verjuice was directly recorded (% or °Brix) using approximately 0.5 mL juice sample.



➔ (U) verjuice (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 th week)

➔ (UV + MH) verjuice (0, 2, 4, 6, 8, 10, 12th week)

➔ (P) verjuice (0, 2, 4, 6, 8, 10, 12 th week)

MICROBIOLOGICAL ANALYSIS

Total mesophilic counts (log CFU/mL)
Yeasts and moulds (log CFU/mL)
Coliforms (log CFU/mL)

PYSICOCHEMICAL & OPTICAL ANALYSIS

pH	Absorption coefficient (cm^{-1})
TSS (°Brix)	Color (L*, a*, b*, ΔE, BI)
Titratable acidity (%)	Turbidity (NTU)

Figure 4.3. Diagram of the shelf life study of freshly squeezed verjuice

4.1.5.3. Titratable acidity

Titratable acidity was expressed as percent tartaric acid. Tartaric acid is a characteristic of grapes; its content ranges from 4-6 g/L in mature grapes, to around 15 g/L in unripe grapes (Scientific, 2018). Titratable acidity was measured by titrating the verjuice with 0.1 N standardized sodium hydroxide (NaOH) solution with the phenolphthalein indicator until reaching the end point (AOAC, 1984). The endpoint (conversion of color) is reached at pH 8.1. The amount of NaOH spent was recorded. The percent of titratable acidity (%) was determined according to the Equation 4.5.

$$TA\ (\%) = (V) * (f) * (E) * 100\ /\ M \quad (4.5)$$

where V is the volume of 0.1 N NaOH during titration (mL), f is normality factor (0.9158 g), E is milliequivalent weight of tartaric acid for 0.1 N NaOH (0.007505 g) and M is the volume of the verjuice sample used in the titration (5mL).

4.1.5.4. Absorption coefficient

The absorption coefficient of verjuice samples was determined from the measurement of absorbance values by means of a Carry 100 UV-Visible Spectrophotometer (Varian Inc., CA, USA) set at a wavelength of 254 nm. Several dilutions of verjuice were made in volumetric flasks with distilled water in the range of 1:10, 1:25, 1:50, 1:100, 1:200, and 1:500. The spectrophotometer was firstly zeroed with distilled water. Each diluted verjuice samples were placed in one-centimeter square quartz cuvettes and read against water at 254 nm wavelength. The absorbance values versus concentration of samples were plotted. The slope of the graph is the absorbance coefficient (cm^{-1}) of the verjuice.

4.1.5.5. Turbidity

A turbidimeter (Model 2100AN IS, HACH Company, USA) was used to measure the amount of cloudiness of the verjuice. Thirty milliliters of verjuice sample was placed

in the glass tube of the equipment and the turbidity was recorded in terms of Nephelometric Turbidity Unit (NTU) within 2 or 3 min.

4.1.5.6. Color

Color parameters of the verjuice were determined by using Konica Minolta CR 400 Chromometer (Konica Inc, Japan) using quartz sample cup. Measurements were expressed as CIE color parameters as L* (lightness-darkness), a* (redness-greenness), b* (yellowness-blueness). L* value indicates lightness and it changes from 0 (dark) to 100 (light); a* value is the color change from green (-) to red (+) and its value increases towards red; b* value represents the color change from blue (-) to yellow (+) and high b* indicates more yellowness (CIE, 1995). Hue angle and chroma that are related to a* and b* values were also determined. Hue angle (h°) at 0°, 90°, 180° and 270° represents redness, yellowness, greenness, and blueness, respectively. The chroma value (C*) is responsible for the color intensity or saturation of the sample (Gejima, Zhang & Nagata, 2003; Pathare, Opara, & Al-Said, 2013). A three-dimensional image of the L*, a*, b*, h° and C* values were shown in Figure 4.4. Hue angle and chroma value were calculated from Equation 4.6 and 4.7 Besides these parameters, the total color difference (ΔE) and browning index (BI) of verjuice samples were calculated from the Equation 4.8 and 4.9.

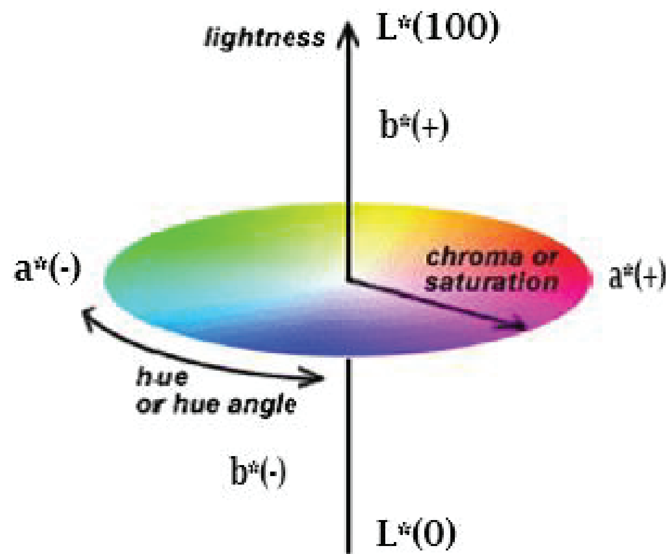


Figure 4.4. CIELAB color space
(Pathare et al., 2013)

$$\text{Hue angle } (h°) = \tan^{-1}(b^* / a^*) \quad (4.6)$$

$$\text{Chroma}(C^*) = \sqrt{(a*)^2 + (b*)^2} \quad (4.7)$$

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (4.8)$$

$$\text{BI} = 100 * \frac{\frac{(a* + 1{,}75\, L*)}{(5{,}645\, L* + a* - 3{,}012\, b*)} - 0.31}{0{,}172} \quad (4.9)$$

4.1.6. Data Analysis

All of the pasteurization processes were repeated three times. The results of microbiological, physicochemical and optical analyses of verjuice samples were expressed as their means and standard deviations. Measurements were done in triplicate. Regression analysis was applied by using a commercial spreadsheet (Microsoft Excel, Redmond, WA, USA). One-way analysis of variance (ANOVA) was carried out to determine how significantly the independent variables (U, UV+MH2 and P processes) affect the dependent variables (microbiological, physicochemical and optical properties of verjuice) by using the Minitab 16 software program (Minitab Inc., State College, PA, USA). The means were evaluated in terms of Tukey comparison test with a 95% confident interval. Differences of data were significant for p-value is equal or less than 0.05 ($p \leq 0.05$).

4.2. Results and Discussion

4.2.1. Effect of Combined UVC-Mild Heat (UV+MH2) Treatment on the Quality Properties of Verjuice

The effect of combined UV-C-mild heat (UV+MH2) treatment at a UV dose of 77.98±0.26 mJ/mL and 51.24±2.74 °C, and conventional thermal pasteurization (P)

applied at 72 °C for 18 s on the physicochemical and optical properties of verjuice are provided in Table 4.2 and 4.3. pH (2.61-2.62), titratable acidity (2.95-2.97%), total soluble solids content (4.13 °Brix) and turbidity (28.10-30.67 NTU) of processed and unprocessed verjuice did not show any significant differences (p>0.05). These data were in agreement with other studies reporting no significant changes in pH, total soluble solids content, titratable acidity and turbidity of fruit juices treated with UV-C light and heat treatment (Caminiti et al., 2011a; Muller, Noack, Greiner, Stahl, & Posten, 2014; Noci et al., 2008; Pala & Toklucu, 2013b; Santhirasegaram, Razali, George, & Somasundram, 2015).

Similarly, the absorption coefficients of the untreated (U) (27.14 cm^{-1}) and UV+MH2 treated samples (27.43 cm^{-1}) were not statistically different from each other (p>0.05). However, the absorption coefficient of thermally pasteurized verjuice (26.61 cm^{-1}) was lower than the U and UV+MH2 treated samples (Table 4.2). Although the ascorbic acid content of the samples was not measured in this study, Koutchma (2008) reported that the degradation of ascorbic acid (vitamin C) was the reason for a decrease in the absorbance of fruit juices. They reported the juice enriched with vitamin C had higher absorption coefficient. Additionally, vitamin C is also very sensitive to heat and oxygen (Robertson & Samaniego, 1986). The thermal effect was also the cause of degradation of vitamin C in verjuice at 72°C resulting in a cloud loss.

Table 4.2. Physicochemical and optical properties of untreated (U), treated with combined UV-C and mild heating (UV+MH2) and thermally pasteurized (P) verjuice.

Treatment	**pH**	**TSS (°Brix)**	**TA (%)**	**Turbidity (NTU)**	**Abs. Coef. (cm^{-1})**
U	2.61 ±0.01a	4.13 ±0.06a	2.97 ±0.02a	29.40 ±1.31a	27.14 0.07ab
UV+MH2	2.62 ±0.01a	4.13 ±0.06a	2.97 ±0.00a	30.67 ±1.99a	27.43 0.25a
P	2.62 ±0.01a	4.13 ±0.06a	2.95 ±0.02a	28.10 ±1.51a	26.61 0.23b

Results were presented as “means± standard error”. The least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different (p≤0.05). TSS: Total Soluble Solid content, TA: Titratable acidity, Abs. coef.: Absorption coefficient.

Color parameters of verjuice were noticeably changed after combined UV+MH2 and thermal (P) processes (Table 4.3 and Figure 4.5). The L* value (lightness–darkness)

of the verjuice (29.68) was significantly decreased; the juice became slightly darker by the UV+MH2 (28.91), whereas it was not changed by the thermal treatment (29.57). Aguilar, Ibarz, Garvin, and Ibarz (2016) also reported the loss of lightness in nectarine juice after UV-C irradiation at 45 °C. They interpreted that moderate temperatures causes browning of the juice. Mansor, Shamsudin, Adzahan, and Hamidon (2017) also reported lower L* value or darker color of the pineapple juice after UV-C irradiation by the effect of mild heat. a*(redness-greenness) and b*(yellowness-blueness) values of the verjuice were significantly increased after treatments of UV+MH2 and P ($p<0.05$). Bhat and Stamminger (2015) also reported an increase of a* value in freshly squeezed strawberry juice. This was explained by the generation of brown color melanoidin pigments. It was reported that unripe grape has carotenoids responsible from yellow or red color (Kamffer, Bindon, & Oberholster, 2010). Yellowness was also markedly detected in verjuice samples since b* values were significantly increased after treatments. Higher b* value in verjuice could be due to possible enhancement of carotenoid types of compounds in the juice composition (Bhat & Stamminger, 2015). Similarly, Muller et al. (2014) found lower L*, higher a*, and b* values after UV-C irradiation of apple and grape juice. Their results indicated that browning reactions were favoured in the juice as a result of the oxidation of phenolic compounds by active PPO enzymes and the high amount of oxygen available during the UV-C treatment.

Chroma value (C*) showed the saturation or color intensity of verjuice. It was observed that color intensity of UV+MH2 treated verjuice (C*:5.94) was significantly higher than P verjuice (C*: 3.16) (Table 4.3). Similarly, Chia et al. (2012) reported higher chroma value in UV-C treated pineapple juice compared to thermally pasteurized juice, i.e. juice became more intense color by UV-C irradiation. Similarly, UV-C treated pineapple juice was more yellow with a higher hue angle (h°) in comparison to thermally pasteurized juice (Chia et al., 2012). However, table 4.3 showed that hue angle of UV+MH2 treated and thermal pasteurized verjuice was slightly lower in comparing to untreated juice, i.e. the color was turned to the red according to the scale given by Pathare et al. 2003. The reason of lower hue angle could be related to the browning reactions occurred in the juice after heat treatments (Lozano & Ibarz, 1997).

The total color difference (ΔE) of verjuice was classified as unnoticeable (0-0.5), slightly noticeable (0.5-1.5), noticeable (1.5-3.0), well visible (3.0-6.0) and great (6.0-12.0) according to Cserhalmi, Sass-Kiss, Toth-Markus, and Lechner (2006). Based on this classification, the ΔE value of P treated samples were slightly noticeable (0.24),

whereas UV+MH2 treated samples were noticeable (3.09) (Figure 4.5 & Figure A.2). A significant increase in the browning index in UV+MH2 (BI: 22.98) and P (BI: 11.20) treated verjuice indicated that all treatments caused a darkening of verjuice (Figure 4.5). Zhang et al. (2011) reported an increase of browning degree of the watermelon juice by the effect of UV-C irradiation and thermal treatment. Aguilar et al. (2016) reported a change in the composition of sugars in the juice after the UV-C treatment. This was attributed to heating and darkening reactions i.e., Maillard, caramelization or HMF formation.

Table 4.3. Color properties of untreated (U), treated with combined UV-C and mild heating (UV+MH2) and thermally pasteurized (P) verjuice.

Treatment	**L***	**a***	**b***	**h°**	**C***
U	29.68 ±0.02a	0.08 0.01c	2.95 ±0.02c	88.49 ±0.28a	2.95 ±0.02c
UV+MH2	28.91 ±0.05b	0.31 0.01a	5.93 ±0.05a	86.99 ±0.10c	5.94 ±0.05a
P	29.57 ±0.07a	0.12 0.01b	3.16 ±0.02b	87.76 ±0.18b	3.16 ±0.02b

Results were presented as “means± standard error”. The least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different (p≤0.05). TSS: Total Soluble Solid content, TA: Titratable acidity, Abs. coef.: Absorption coefficient.

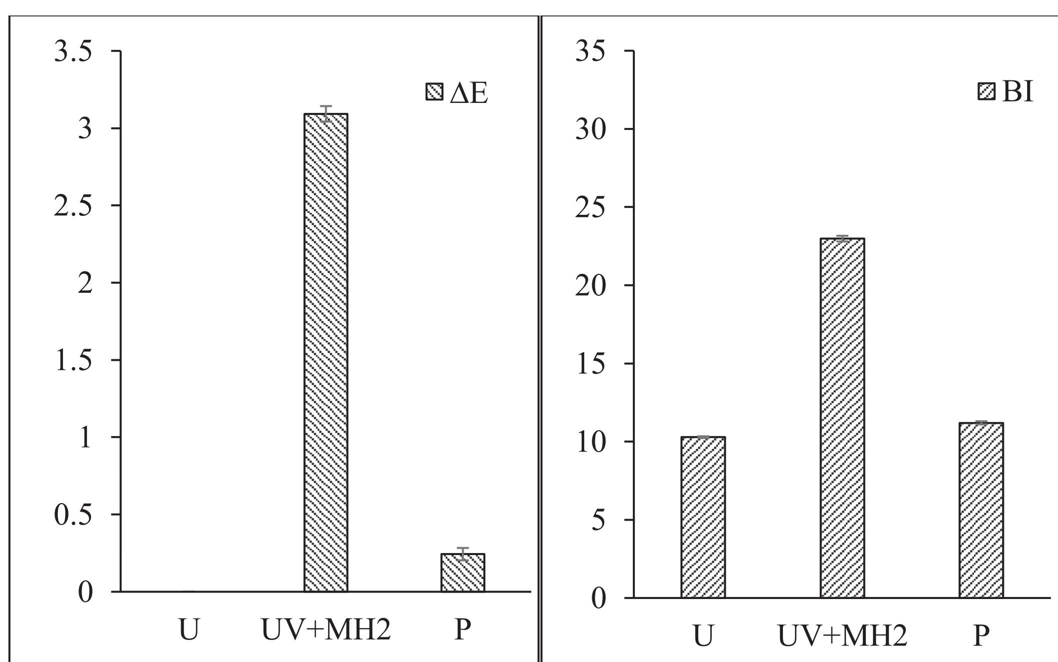


Figure 4.5. Total color difference (ΔE) and browning index (BI) of untreated (U), treated with combined UV-C and mild heating (UV+MH2) and thermally pasteurized (P) verjuice

4.2.2. Effect of Combined UV+MH2 Treatment on the Quality Properties of Verjuice During Storage

4.2.2.1. Microbiological Properties of Verjuice

The fruit juice and nectars should comply with microbiological criteria established in accordance with the Principles for the Establishment and Application of Microbiological Criteria for Foods (CAC/GL 21-1997), Institute of Food Science and Technology (1999) and Turkish Food Codex (the Microbiological Criteria, No: 2001/19).The acceptable maximum TMAC (total mesophilic aerobic counts) and YM (yeast and molds) counts in fruit juice and nectars must be 4 and 3 log CFU/mL, respectively (IFST, 1999; Turkish Food Codex, 2002). Thus, microbiological spoilage of verjuice samples during shelf life period was evaluated based on these microbiological criteria.

Logarithmic changes in total mesophilic aerobic counts (TMAC), yeast and mold (YMC), and total coliform (TC) counts of verjuice which was untreated (U), treated with combined UV-C+mild heating (UV+MH2) and thermal pasteurization (P) were evaluated during 12 weeks of refrigerated storage (4.22±1.15 °C). Logarithmic changes in yeast and mold, total mesophilic aerobic and coliform bacteria counts of untreated and treated verjuice samples during storage at 4 °C are shown in Table 4.4 and Figure 4.6. Untreated verjuice was completely spoiled by yeast and molds at the end of the 10 weeks (YMC reached to higher than 3 logs (3.02±0.41 log CFU/mL) (Figure 4.6). Verjuice treated with combined UV+MH2 (77.98±0.26 mJ/mL or 19.49±0.07 mJ/cm^2 UV dose, 6 cycles, 496 s, 3.80 mL/s, 51.24±2.74 °C) and thermal pasteurization (P: 72 °C, 18 s) exhibited no microbial growth during 12 weeks of refrigerated storage (Table 4.4). It is concluded that the proposed UV+MH2 treatment is an effective pasteurization method that can be used to extend the shelf life of verjuice products. Similarly, the shelf life of white grape juice subjected to UV dose of 9.95 J/cm^2 (32.5 min) and lemon melon juice blend treated with 2.46 J/mL (16.3 min) using the same continuous flow UV system were extended by more than fifteen-fold and two-fold after UV-C treatment (Kaya et al., 2015; Unluturk & Atilgan, 2015). The findings of this study were in line with the previous studies carried out using the same system, although the applied UV doses were different.

Table 4.4. Microbial quality of verjuice which was untreated (U), and treated with combined (UV+MH2) and thermal pasteurization (P) during storage

	Total mesophilic aerobic counts			**Yeasts & Moulds**			**Coliforms**		
Week	U	UV+MH	P	U	UV+MH	P	U	UV+MH	P
0	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
2	0.17±0.4	-	-	0.17±0.4	-	-	-	-	-
3	0.33±0.52	-	-	0.33±0.52	-	-	-	-	-
4	1.18±0.99	-	-	0.6±0.7	-	-	-	-	-
5	1.04±1.15	-	-	0.94±1.04	-	-	-	-	-
6	1.59±1.28	-	-	2.28±0.83	-	-	-	-	-
8	1.80±0.81	-	-	2.48±0.46	-	-	-	-	-
10	1.97±0.14	-	-	**3.02**±0.41	-	-	-	-	-
12	Spoiled	-	-	Spoiled	-	-	-	-	-

Results were presented as mean±standard error (n = 3).

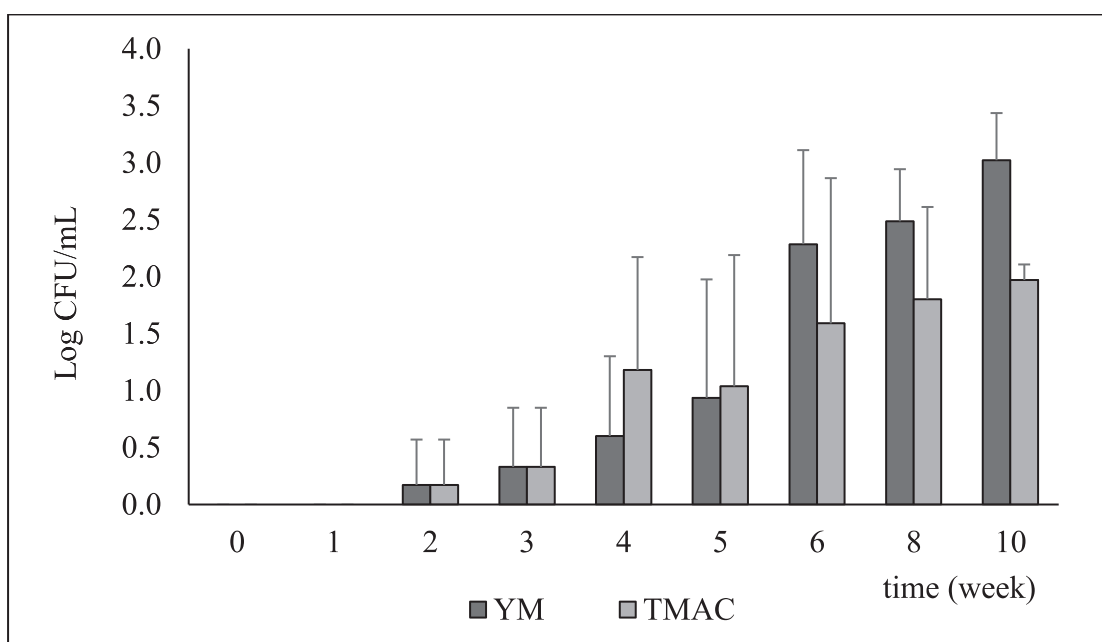


Figure 4.6. Changes in yeasts and molds (YM), and total mesophilic aerobic counts (TMAC) in (freshly squeezed verjuice (U) during storage

Several studies reported that the shelf life of fruit juices stored under refrigerated conditions (4 °C) was extended for a few more days by applying UV-C irradiation (Feng, Ghafoor, Seo, Yang & Park, 2013; Kaya et al., 2015; La Cava & Sgroppo, 2015; Muller et al., 2014; Pala & Toklucu, 2013b; Santhirasegaram et al., 2015; Tran & Farid, 2004;

Unluturk & Atilgan, 2015). La Cava and Sgroppo (2015) monitored the change of total mesophilic aerobic counts (TMAC) and yeasts and molds (YM) in grapefruit juice treated with 3.94 J/cm^2 UV dose at refrigerated temperature (4°C). They reported that the shelf life of grapefruit juice was delayed 10-15 days by using UV-C irradiation; no microbial growth was detected in UV-C treated grapefruit juice until 10 days, whereas, untreated (control) juice contained 2.02 and 2.61 log CFU/mL TMAC and YM, respectively at the end of the 10 days. Similarly, Pala and Toklucu (2013b) found that freshly squeezed orange juice treated with UV-C irradiation had a longer shelf life during storage compared to untreated juice. They were able to extend the shelf life of UV-C irradiated orange juice up to 5 days and 9 days by storing at 10 °C and 4 °C, respectively. Tran and Farid (2004) extended the shelf life of freshly squeezed orange juice up to 5 days by applying 73.8 mJ/cm^2 UV dose. Feng et al. (2013) evaluated the microbiological quality of untreated and UV-C treated watermelon juice (exposed to UV dose of 2.7 and 37.5 J/mL) at 5 °C. They observed that untreated watermelon juice was reached to unacceptable level within 14 days due to the growth of yeasts, molds, and bacteria (>6 log CFU/mL). However, they reported that the juice treated with 37.5 J/mL was consumable until the end of the 31 days of storage at 5 °C. They also noticed that the microbial growth in watermelon juice exposed to the lower UV dose (2.7 J/mL) occurred faster than the juice exposed to the higher UV dose (37.5 J/mL). Muller et al. (2014) applied 100 kJ/L UV dose to apple and grape juice and monitored their microbial deteriorations at 4 °C storage conditions. Untreated apple and grape juices were reached to 7 log CFU/mL microbial count after 15 and 18 days, respectively. On the other hand, the microbial load of UV-C treated juices were around 5 log CFU/mL after 18 days. Santhirasegaram et al. (2015) monitored the shelf life of UV-C treated mango juice at refrigerated temperature during 5 weeks of storage period. In their study, the shelf life of mango juice treated with 15, 30 and 60 min of UV-C irradiation (with 3.525 J/m^2 UV intensity) was determined as 2, 3-4 and 5 weeks respectively, while untreated juice was immediately spoiled within less than 1 week based on the microbiological criteria depicted by IFST (1999).

Kaya et al. (2015) used the same continuous flow UV system to process the lemon melon juice blend. They also observed that the shelf life of LMJ blend treated with 2.461 J/mL UV dose was extended by more than 30 days under the refrigerated conditions (4.0±0.82 °C), while the untreated ones were completely spoiled within 2 days. Unluturk and Atilgan (2015) also monitored the microbiological quality of untreated and UV-C pasteurized (9.92 J/cm^2) freshly squeezed white grape juice during refrigerated storage.

They reported that UV-C treated white grape juice was shelf-stable up to 14 days at 4 °C, while untreated juice became inconsumable within 7 days by growing of yeasts and lactic acid bacteria.

In conclusion, the refrigerated shelf life of verjuice was successfully extended more than 12 weeks as a result of exposure to combined UV-C and mild heating. On the other hand, untreated fresh verjuice was completely spoiled in 10 weeks under the same conditions.

4.2.2.2. Physicochemical and Optical Properties of Verjuice during Storage

Physicochemical and optical properties of untreated and treated verjuice samples right after the treatments and during the refrigerated storage (4.22±1.15 °C) period were shown in Table 4.5, Table 4.6, Figure 4.7 and Figure 4.8. One-way ANOVA analysis was applied to the results of physicochemical and optical analyses of verjuice samples. The data were assessed by Tukey comparison test at a 95% confidence interval.

There were no significant differences in pH, total soluble solid content (TSS) and titratable acidity (TA) values after UV-C treatment compared to untreated samples ($p>0.05$). Other studies in the literature showed no significant changes in pH, soluble solids and titratable acidity values of juices after UV-C treatment (Canitez, 2002; Chia et al., 2012; Donahue, Canitez, & Bushway, 2004; Feng et al., 2013; Kaya et al., 2015; La Cava & Sgroppo, 2015; Manzocco, Quarta, & Dri, 2009; Muller et al., 2014; Riganakos, Karabagias, Gertzou, & Stahl, 2017; Tandon, Worobo, Churey, & Padilla-Zakour, 2003; Torkamani & Niakousari, 2011; Unluturk & Atilgan, 2015).

pH of all treated and untreated verjuice was not different from each other (in the range of 2.60 and 2.62) during 12 weeks storage period at refrigerated conditions. Thus, the storage time had no effect on pH of untreated, UV+MH2 treated and thermally pasteurized verjuice samples (Figure 4.7a). Similarly, Donahue et al. (2004) and Riganakos et al. (2017) found no significant changes in pH of UV-C treated apple cider, and UV-C treated and thermally pasteurized carrot juice during storage.

Table 4.5. Changes in physicochemical properties of verjuice during 12 weeks of refrigerated storage

Week	**Treatment**	**pH**	**TSS (°Brix)**	**TA (%)**	**Turbidity (NTU)**	**Abs. coef. (cm^{-1})**
0	**U**	2.61 ±0.01*ab*	4.13 ±0.06*a*	2.97 ±0.02ab	29.40 ±1.31*a*	27.14 ±0.07*a*
	UV+MH2	2.62 ±0.01A	4.13 ±0.06A	2.97 ±0.00A	30.67 ±1.99A	27.43 ±0.25A
	P	2.62 ±0.01a	4.13 ±0.06a	2.95 ±0.02a	28.10 ±1.51a	26.61 ±0.23a
1	**U**	2.60 ±0.02*ab*	4.17 ±0.06*a*	3.01 ±0.01a	28.63 ±1.17*a*	27.56 ±0.46*a*
2	**U**	2.62 ±0.01*a*	4.10 ±0.00*a*	2.92 ±0.03b	33.87 ±8.99*ab*	27.80 ±0.45*a*
	UV+MH2	2.61 ±0.01A	4.10 ±0.00A	2.92 ±0.03A	55.17 ±1.91AB	28.54 ±0.55B
	P	2.61 ±0.01a	4.13 ±0.06a	2.93 ±0.02a	36.87 ±2.00b	27.85 ±0.84ab
3	**U**	2.61 ±0.01*ab*	4.20 ±0.12*a*	2.93 ±0.03ab	45.07 ±3.52*bc*	28.25 ±0.47*a*
4	**U**	2.62 ±0.01*a*	4.20 ±0.10*a*	2.93 ±0.04b	50.37 ±1.22*c*	27.87 ±0.07*a*
	UV+MH2	2.62 ±0.01A	4.13 ±0.06A	2.94 ±0.02A	66.23 ±4.86BC	28.66 ±0.55B
	P	2.61 ±0.01a	4.10 ±0.00a	2.94 ±0.02a	43.70 ±5.86b	27.98 ±0.44ab
5	**U**	2.60 ±0.00*b*	4.17 ±0.06*a*	2.93 ±0.01b	52.73 ±4.05*cd*	28.34 ±0.69*a*
6	**U**	2.62 ±0.01*ab*	4.10 ±0.00*a*	2.96 ±0.03ab	54.30 ±1.97*cd*	28.13 ±0.71*a*
	UV+MH2	2.62 ±0.01A	4.17 ±0.06A	2.94 ±0.02A	104.8 ±7.35D	28.40 ±0.17AB
	P	2.62 ±0.01a	4.23 ±0.06a	2.92 ±0.02a	67.43 ±1.42c	28.15 ±0.55b
8	**U**	2.62 ±0.01*ab*	4.10 ±0.00*a*	2.95 ±0.02ab	55.67 ±3.39*cd*	28.26 ±0.36*a*
	UV+MH2	2.61 ±0.01A	4.10 ±0.00A	2.94 ±0.02A	87.83 ±8.14CD	29.36 ±0.44BC
	P	2.60 ±0.01a	4.17 ±0.06a	2.94 ±0.01a	62.53 ±2.58c	28.73 ±0.28b
10	**U**	2.60 ±0.01*b*	4.10 ±0.10*a*	2.95 ±0.03ab	62.53 ±5.01*d*	27.76 ±0.61*a*
	UV+MH2	2.61 ±0.01A	4.10 ±0.00A	2.96 ±0.02A	104.3 ±4.16D	29.86 ±0.29C
	P	2.60 ±0.01a	4.17 ±0.06a	2.96 ±0.01a	69.0 ±2.67c	29.10 ±0.75b
12	**UV+MH2**	2.60 ±0.01A	4.03 ±0.12A	2.96 ±0.03A	135.7 ±21.2E	29.41 ±0.29BC
	P	2.61 ±0.01a	4.10 ±0.00a	2.94 ±0.01a	84.67 ±2.05d	28.21 ±0.35b

Results were presented as mean±standard deviations (n = 3). Tukey least significant difference test was applied ($p \leq 0.05$). Different letters for each process show the differences in properties between weeks during shelf life study. *İtalic lower case letters (a,b)*: untreated samples; CAPITAL LETTER (A, B): UV-treated samples; lower case letters (a,b): thermally treated samples

Total soluble solid content (TSS) of untreated (U), UV+MH2 treated and thermally pasteurized (P) verjuice were not significantly changed throughout the storage period ($p<0.05$). TSS values were in the range of 4.1-4.2 °Brix (Figure 4.7b). Feng et al. (2013) reported that storage time of 37 days did not have a significant impact on TSS content of untreated and UV-C treated (2.7 J/mL and 37.5 J/mL) watermelon juice during storage period at 5±1 °C. Torkamani and Niakousari (2011) also found that TSS content of orange juice exposed to 125 mJ/cm^2 UV dose did not change after 7 days. Tandon et al. (2003) also monitored TSS content of UV-C (14 mJ/cm^2) treated and hot filled pasteurized (63 °C) apple cider and they did not observe any variation in TSS during 14 weeks of refrigerated storage.

Titratable acidity values of U, UV+MH2 treated and P verjuice were not significantly affected from the storage time, i.e. tartaric acid- based acidity values were between 2.90 and 3.00% during 12 weeks of storage period (Figure 4.7c). Similarly, La Cava and Sgroppo (2015) observed no change in titratable acidity content of untreated and UV-C treated grapefruit juice during 30 days of storage at both 4 °C and 10 °C. Besides, Kaya et al. (2015) reported no considerable change in the titratable acidity of UV-C treated and thermally pasteurized Lemon Melon Juice blend during storage (30 days at 4.0±0.82 °C). Titratable acidity level was not changed during storage period, supporting the results obtained for pH (Table 4.5).

Absorption coefficient slightly increased both in the treated and untreated juice samples during 12 weeks of storage (Figure 4.7d). This was probably due to the breakdown of color compounds in fruit juices during storage, and then the formation of melanin and melanoidin pigments by browning reactions (Unluturk & Atilgan, 2015). Similarly, Muller et al. (2014) found an increase in the absorption coefficient of UV-C treated apple and grape juices. They ascribed this to browning reactions formed due to UV-C irradiation. The literature has reported the absorptivity property of melanoidin pigments during UV-C inactivation of various enzymes (Seiji & Iwashita, 1965). The results of the experiments revealed that the formation of melanoidins occurred by long exposure of the fruit juice samples to UV-C light (Ibarz, Pagan, Panades, & Garza 2005; Seiji & Iwashita, 1965).

On the other hand, turbidity values of untreated and treated verjuice significantly increased during storage ($p\leq0.05$) (Table 4.5 and Figure 4.7e). But the turbidity in untreated and thermally pasteurized (P) samples was significantly ($p<0.05$) less than turbidity of verjuice subjected to UV+MH2 treatment. One possible reason for an increase

in turbidity of untreated and treated verjuice samples might be the formation of tartrate crystals (sodium/potassium bitartrates) from tartaric acids during cold storage (Andres, Riera, & Alvares, 1997; Cemeroglu, 2004). Besides, it was speculated that yeast and mold development during storage could be a reason for an increase in turbidity of the U samples. Unluturk and Atilgan (2015) also pointed out a remarkable increase in the cloudiness of UV-C treated and untreated white grape juice after 14 days of storage. They ascribed this change in turbidity to an increase in microbial count of juice samples from the beginning of the storage period. The increase in cloudiness and sedimentation of juices were also attributed to an increase of microbial growth in the apple cider (Canitez, 2002), pineapple juice (Chia et al., 2012) and LMJ blend (Kaya et al., 2015). Additionally, an increment of turbidity of UV+MH2 and P verjuice samples during storage could also be explained by the formation of protein and polyphenol complexes in the juice composition (Lee, Yusof, Hamid, & Baharin, 2007). The haze in fruit juices can occur due to binding of haze active proteins (contain proline) and haze active polyphenols (hydroxyl groups on the aromatic ring) by means of hydrophobic interactions and hydrogen bonding (Siebert, 1999).

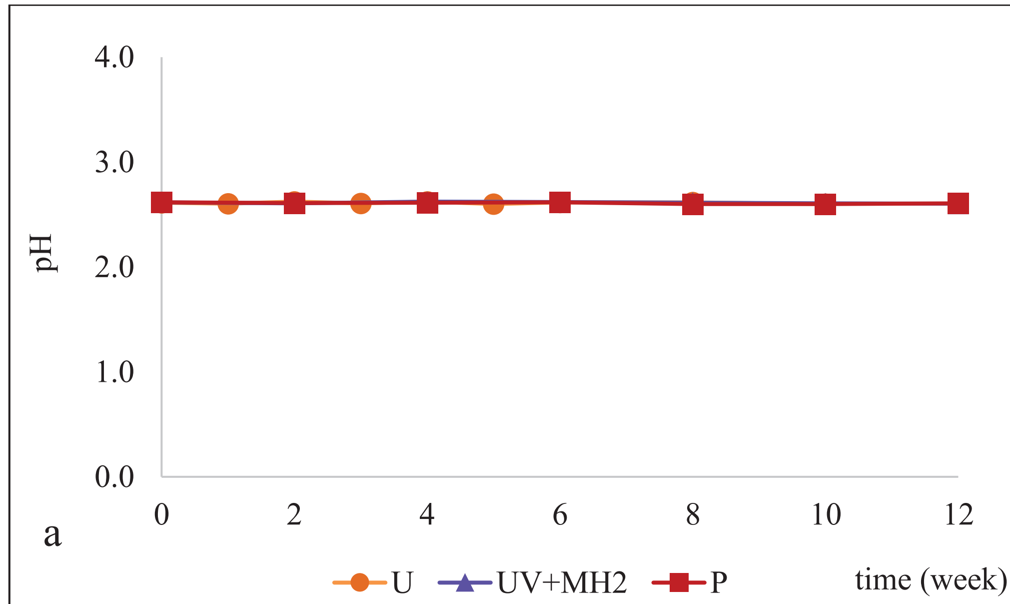
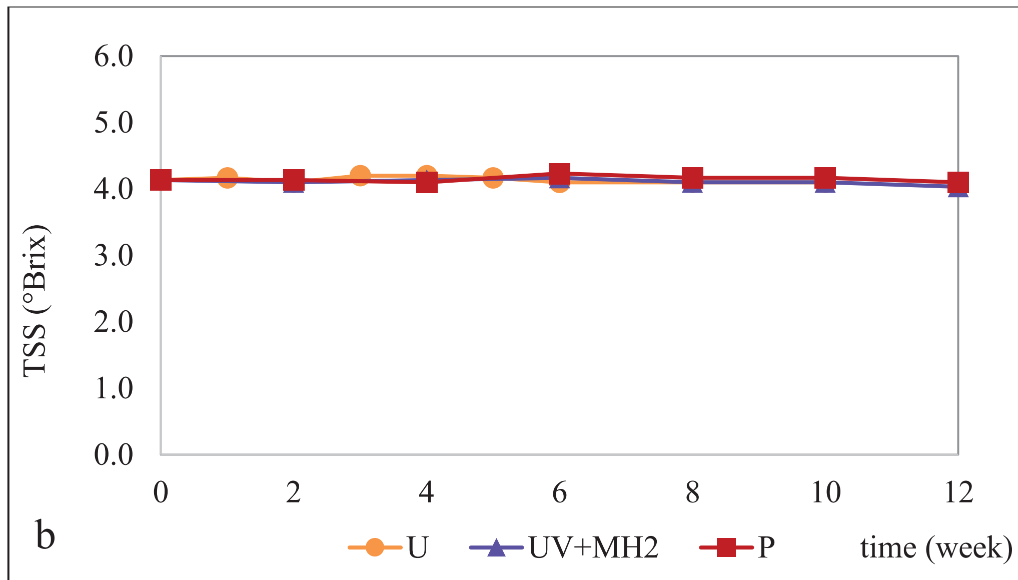
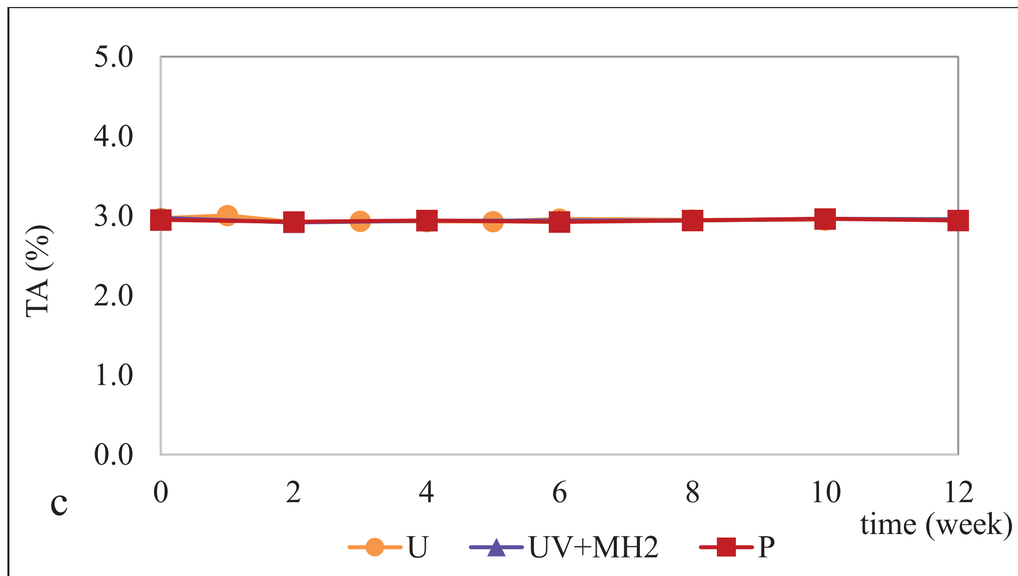


Figure 4.7. Changes in physicochemical properties of untreated (U: circle), combined treated (UV+MH2: triangle) and thermally pasteurized (P: square) verjuice during 12 weeks at refrigerated storage (a: pH, b: Total soluble solid content, c: Titratable acidity, d: Absorption coefficient and e: Turbidity)

(cont. on next page)





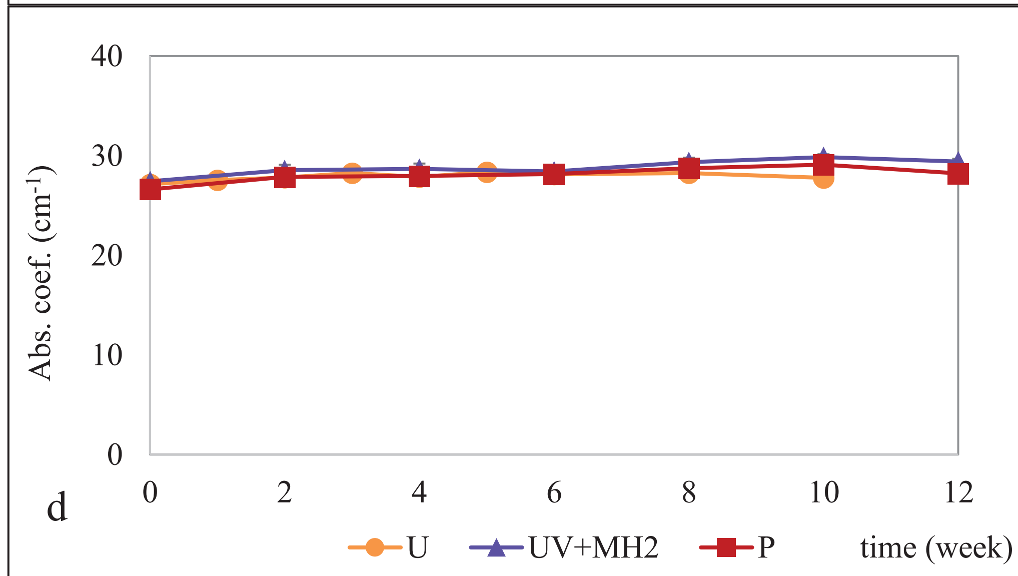


Figure 4.7 (cont.)

(cont. on next page)

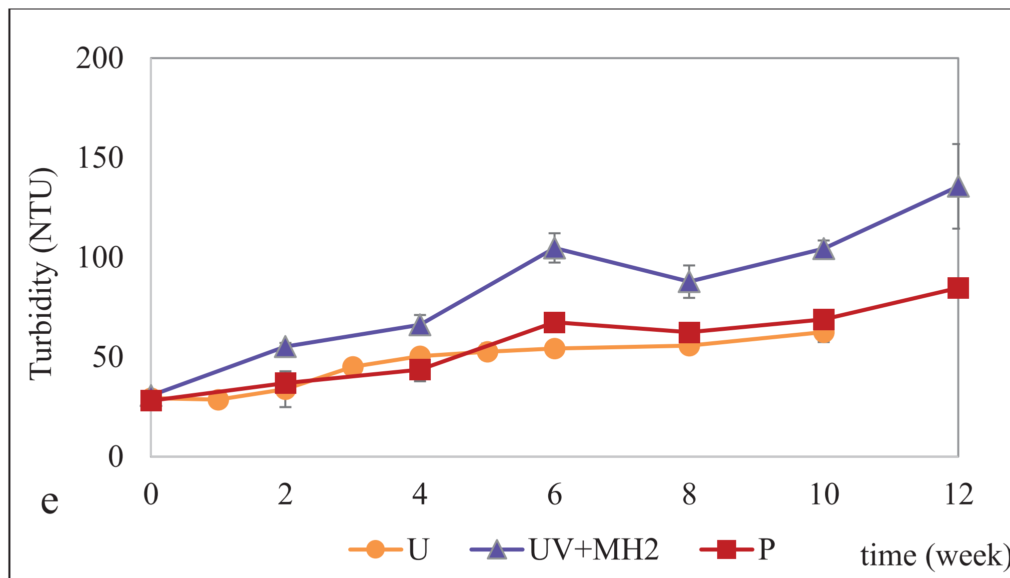


Figure 4.7 (cont.)

It has been well established that the level of pleasantness or unpleasantness of a food product is affected by the intensity of food-related sensory attributes (Cardello, 1994). Color is an important quality attribute of a food product which is directly related to consumer acceptance. Consumers are primarily influenced by the visual sensation affecting subsequent willingness to accept a product. Color has been shown to have a profound effect on perception and acceptability (Imram, 1999). The color of foods is also used as an indirect indicator for flavor and pigment concentration in their compositions (Leon, Mery, Pedreschi, & Leon, 2006; Pathare et al., 2013). Thus, the color properties of verjuice are required to be monitored to evaluate its quality during storage. In the present study, significant change was observed in color parameters (L* (lightness-darkness), a*(redness-greenness), b*(yellowness-blueness), hue angle (h°), chroma (C*), total color difference (ΔE) and BI (browning index) of untreated and treated samples ($p < 0.05$) (Table 4.6 and Figure 4.8).

Storage time had a significant ($p \leq 0.05$) effect on the L*, a*, and b* values of both treated and untreated juice samples. L* (lightness-darkness) values slightly decreased from 29.69, 28.91 and 29.57 to 28.29, 25.23 and 27.55 in untreated, UV+MH2 treated and thermal pasteurized samples, respectively, during storage (Figure 4.8a). The result of this study was in good agreement with the others cited in the literature (Ibarz et al., 2005; Muller et al., 2014; Unluturk & Atilgan, 2015).

In contrast to L^* value, a*(redness-greenness) and b*(yellowness-blueness) of untreated and treated verjuice samples varied significantly during the shelf life period. In

other words, storage time had a significant ($p \leq 0.05$) effect on the a* and b* values of both treated and untreated juice samples (Figure 4.8b & c). Thus, verjuice samples lost their green color and became redder at the end of 12 weeks storage period. The loss of green color could be due to the chlorophyll degradation or generation of brown color melanoidin pigments during storage (Bhat & Stamminger, 2015; Weemaes, Ooms, Van Loey, & Hendrickx, 1999). The value of b*, which reflects yellowness, was significantly increased through the end of 12 weeks of storage in all samples ($p \leq 0.05$). An increase in b^* could be related to enhancement of carotenoid type compounds during shelf life (Bhat & Stamminger, 2015). Ibarz et al. (2005) observed an increase in yellowness of juice attributing to higher melanoidin formation. Muller et al. (2014) similarly observed higher a* and b* values in grape and apple juice after 12 days of storage. They stated that this type of increment was due to the enzymatic browning by the residual polyphenoloxidase enzyme activity retained in the juices after UV-C treatment (100.48 kJ/L). Manzocco et al. (2009) also reported an increased level of a* and b* values in UV-C treated apple slices during refrigerated storage and ascribed this to the enzymatic browning pigment formation caused by the residual polyphenoloxidase enzyme activity.

Hue angle and chroma, that are related to a* and b* values, contribute to the evaluation of the visual color (Chia et al., 2012; Patras, Brunton, Da Pieve, Butler, & Downey, 2009). Hue angle is used to define the qualitative difference of a certain colour with reference to grey colour with the same lightness. It was quoted that "A higher hue angle represents a lesser yellow character in the assays" (Pathare et al., 2013). Hue angle (h°) of all untreated and treated verjuice sample did not prominently change during 12 weeks of storage (Figure 4.8d). Hue angle of UV+MH2 treated verjuice was around approximately 87° at the beginning, then it was slightly decreased to 80° at the end of refrigerated storage. This values indicated that verjuice was in yellow color (~90°), then, slightly turned to red color (0°) by decreasing of hue angle during storage. Pathare et al. (2013) expressed the chroma (C^*) as "The quantitative attribute of colourfulness, is used to determine the degree of difference of a hue in comparison to a grey colour with the same lightness. The higher the chroma values, the higher is the colour intensity of samples perceived by humans". The chroma values of all stored verjuice samples showed a gradual increase with time. However, the color intensity of UV+MH2 treated verjuice samples was better-protected compared to untreated and P treated verjuice samples during refrigerated storage (Figure 4.8e). Chia et al. (2012) found that chroma value of the pineapple juice was better maintained after UV-C irradiation in comparison to the

untreated juice during storage. Patras et al. (2009) also reported an increase in the chroma value or color intensity of tomato purees upon the effect of thermal treatment (70 °C for 20 s).

In the present study, significant change was observed in the BI value of untreated and treated samples ($p \leq 0.05$) during storage (Figure 4.8f). This was because significant effect of storage time was observed in the brightness ($L*$), redness ($a*$), and yellowness ($b*$) of the verjuice samples ($p \leq 0.05$) (Table 4.6). Untreated verjuice was the darkest sample because BI value increased by 2.11-fold at the end of the 10 weeks. On the other hand, UV+MH2 treated verjuice was the least browned sample with a BI value increased by 1.50-fold at the end of the 12 weeks. Additionally, the BI value of the thermally pasteurized verjuice increased up to 1.88-fold at the end of 12-week storage. This observation was in line with the study of Unluturk and Atilgan (2015), as they reported that UVC irradiation of white grape juice subjected to UV dose of 9.92 J/cm^2 resulted in a better colour retention ($BI/BI_0 = 1.75$) compared to the untreated juice ($BI/BI_0 = 3.5$) at the end of the refrigerated storage.

According to the classification made by Cserhalmi et al. (2006), ΔE value of untreated and treated samples increased with storage time and became "noticeable" ($1.5 < \Delta E < 3.0$) at the end of storage period (12 w), whereas dramatic changes in ΔE of UV+MH2 treated juice samples were observed throughout the whole storage period (Figure 4.8g and Table 4.6). The total color change in the UV+MH2 treated verjuice samples reached to 3.93 at the end of 12 weeks and showed well visible difference in color as compared to other juice samples. Similarly, the change in the color of fruit juices subjected to UV-C light and thermal treatment and during storage has also been reported by several studies. This change was attributed to the deterioration of color components (photodegradation) and formation of dark color compounds from enzymatic and non-enzymatic browning reactions (Chia et al., 2012; Falguera, Pagan, & Ibarz, 2011; Ibarz et al., 2005; Kaya et al., 2015; Santhirasegaram et al., 2015; Unluturk & Atilgan, 2015).

Table 4.6. Changes in color properties of verjuice during 12 weeks at refrigerated storage

Week	Treatment	Color						
		L*	a*	b*	h°	C*	ΔE	BI
0	**U**	29.68 ±0.2*a*	0.08 ±0.01*a*	2.95 ±0.02*a*	88.49 ±0.28*a*	2.95 ±0.02*a*	0 *a*	10.28 ±0.06*a*
	UV+MH2	28.91 ±0.05A	0.31 ±0.01A	5.93 ±0.05A	86.99 ±0.10A	5.94 ±0.05A	0 A	22.98 ±0.18A
	P	29.57 ±0.07a	0.12 ±0.01a	3.16 ±0.02a	87.76 ±0.18a	3.16 ±0.02a	0 a	11.20 ±0.10a
1	**U**	29.61 ±0.06*a*	0.25 ±0.02*b*	2.96 ±0.02*a*	85.09 ±0.34*bc*	2.97 ±0.02*a*	0.20 ±0.03*ab*	10.79 ±0.11*a*
2	**U**	29.31 ±0.13*b*	0.30 ±0.01*bc*	3.47 ±0.06*b*	85.10 ±0.05*bc*	3.48 ±0.06*b*	0.68 ±0.11*bc*	12.90 ±0.27*b*
	UV+MH2	28.10 ±0.03B	0.61 ±0.01B	6.65 ±0.08B	84.72 ±0.00B	6.68 ±0.08B	1.13 ±0.05B	27.71 ±0.40B
	P	29.29 ±0.01ab	0.25 ±0.02b	3.58 ±0.02b	86.07 ±0.23b	3.59 ±0.02b	0.52 ±0.06b	13.18 ±0.10b
3	**U**	29.04 ±0.11*bc*	0.34 ±0.01*bc*	3.63 ±0.07*bc*	84.67 ±0.15*b*	3.65 ±0.07*b*	0.97 ±0.11*cd*	13.76 ±0.34*bc*
4	**U**	28.85 ±0.13*cd*	0.34 ±0.03*bc*	4.06 ±0.08*cd*	85.16 ±0.28*bc*	4.08 ±0.08*c*	1.42 ±0.11*de*	15.54 ±0.43*c*
	UV+MH2	27.33 ±0.08C	0.89 ±0.04C	6.67 ±0.03B	82.40 ±0.39C	6.73 ±0.02B	1.84 ±0.04C	29.44 ±0.13C
	P	29.13 ±0.21b	0.37 ±0.05c	3.31 ±0.10ab	83.63 ±0.68c	3.34 ±0.10ab	0.54 ±0.26b	12.59 ±0.58b
5	**U**	28.81 ±0.15*cd*	0.37 ±0.06*c*	4.26 ±0.17*ef*	84.99 ±0.57*bc*	4.28 ±0.17*cd*	1.60 ±0.21*e*	16.41 ±0.90*cd*
6	**U**	28.74 ±0.11*de*	0.38 ±0.06*c*	4.58 ±0.21*fg*	85.31 ±0.68*bc*	4.59 ±0.21*d*	1.91 ±0.26*ef*	17.73 ±1.00*d*
	UV+MH2	26.41 ±0.11D	1.01 ±0.03D	6.92 ±0.03C	81.73 ±0.28C	6.99 ±0.17C	2.78 ±0.12D	32.19 ±1.64D
	P	28.07 ±0.10c	0.48 ±0.02d	4.15 ±0.03c	83.41 ±0.20c	4.18 ±0.46c	1.83 ±0.03c	16.69 ±2.23c
8	**U**	28.48 ±0.07*ef*	0.36 ±0.05*bc*	4.79 ±0.08*g*	85.67 ±0.61*bc*	4.80 ±0.08*d*	2.22 ±0.11*f*	18.72 ±0.38*d*
	UV+MH2	26.45 ±0.09D	1.01 ±0.01D	7.28 ±0.12D	82.09 ±0.09C	7.35 ±0.12D	2.89 ±0.03D	33.95 ±0.48E
	P	27.97 ±0.02c	0.49 ±0.01d	4.78 ±0.05d	84.10 ±0.05c	4.81 ±0.05d	2.31 ±0.05d	19.42 ±0.26d
10	**U**	28.29 ±0.11*f*	0.37 ±0.07*bc*	5.47 ±0.35*h*	86.17 ±0.56*c*	5.48 ±0.35*e*	2.90 ±0.39*g*	21.73 ±1.69*e*
	UV+MH2	25.90 ±0.14E	1.21 ±0.01E	7.53 ±0.05E	80.89 ±0.05D	7.63 ±0.05E	3.53 ±0.08E	36.67 ±0.07F
	P	27.94 ±0.03c	0.59 ±0.01e	5.05 ±0.09e	83.36 ±0.11c	5.08 ±0.09e	2.54 ±0.07de	20.81 ±0.42e
12	**UV+MH2**	25.23 ±0.19F	1.18 ±0.05E	6.96 ±0.15C	80.38 ±0.51D	7.05 ±0.15CD	3.93 ±0.15F	34.65 ±0.52E
	P	27.55 ±0.11d	0.57 ±0.03e	5.05 ±0.12e	83.57 ±0.22c	5.08 ±0.12e	2.81 ±0.07e	21.08 ±0.49e

Results were presented as mean±standard deviations (n = 3). Tukey least significant difference test was applied ($p \leq 0.05$). Different letters for each process show the differences in properties between days during shelf life study. *İtalic lower case letters (a,b)*: untreated samples; CAPITAL LETTER (A, B): UV-treated samples; lower case letters (a,b): thermally treated samples.

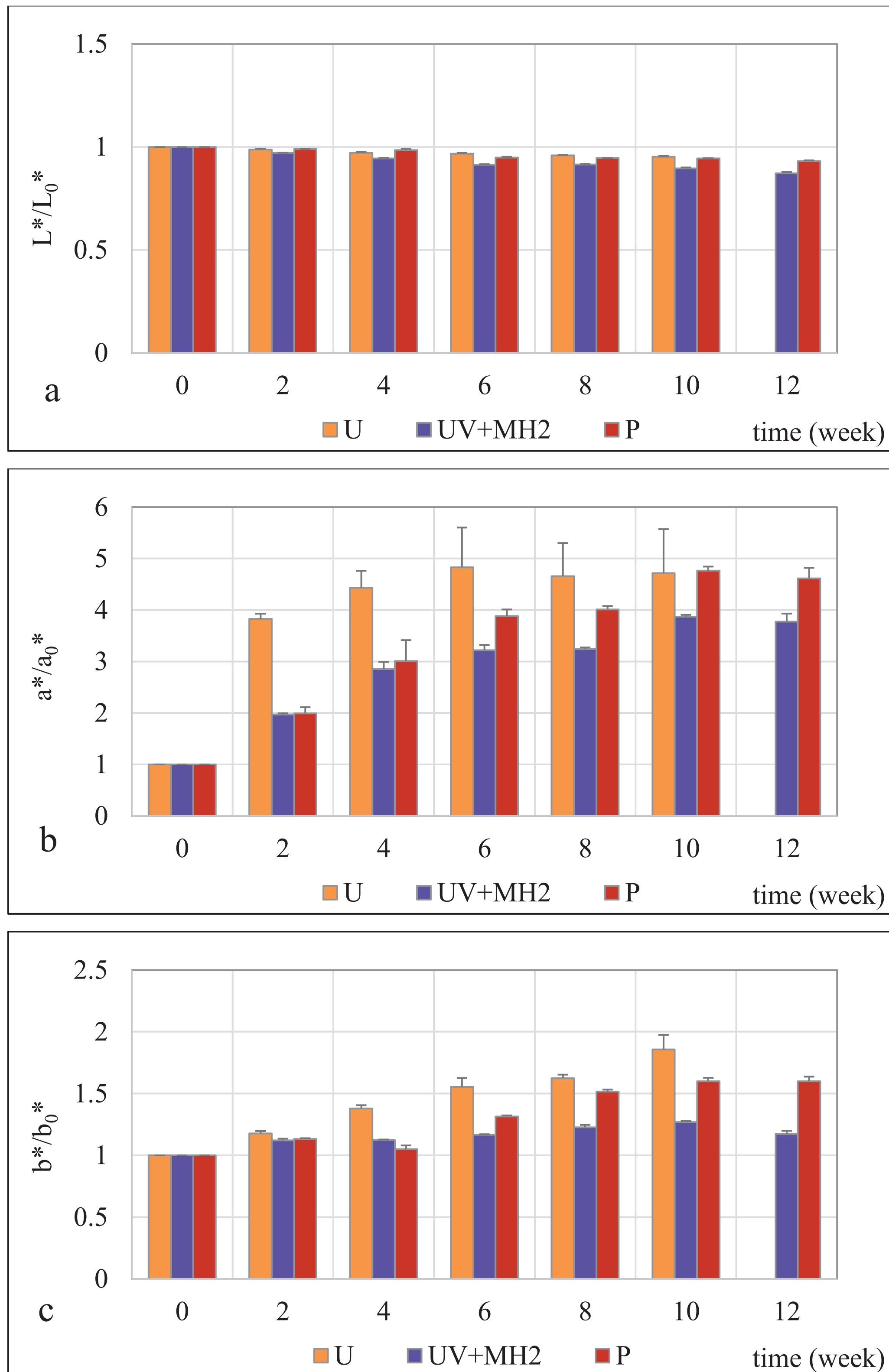
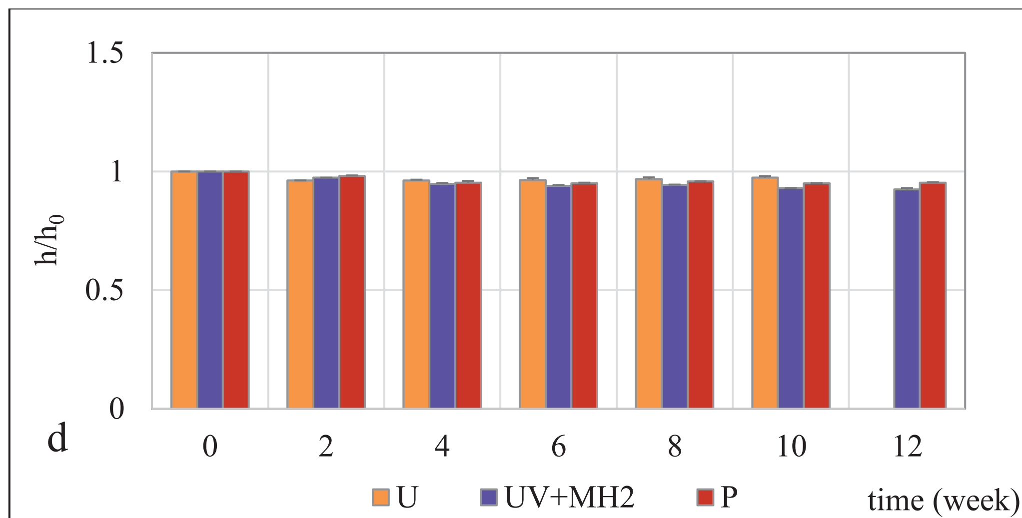
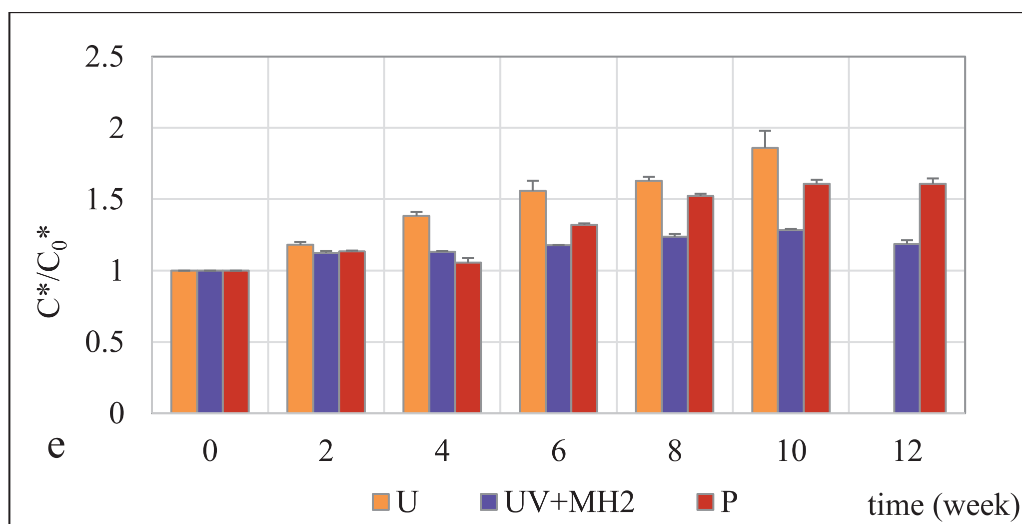


Figure 4.8. Changes in color of untreated (U: circle), treated with combination of processes (UV+MH2: triangle) and thermally (P: square) pasteurized verjuice during 12 weeks at refrigerated storage (a: lightness-darkness, L*, b: redness-greenness a*, c: yellowness-blueness, b*, d: hue angle, h°, e: chroma, C*, f: total color difference, ΔE and g: browning index, BI)

(cont. on next page)





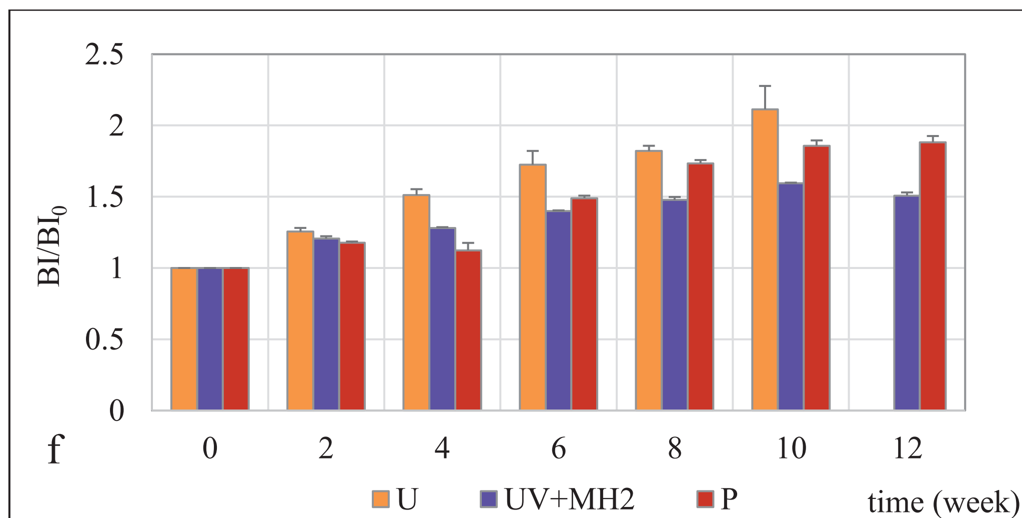


Figure 4.8. (cont.)

(cont. on next page)

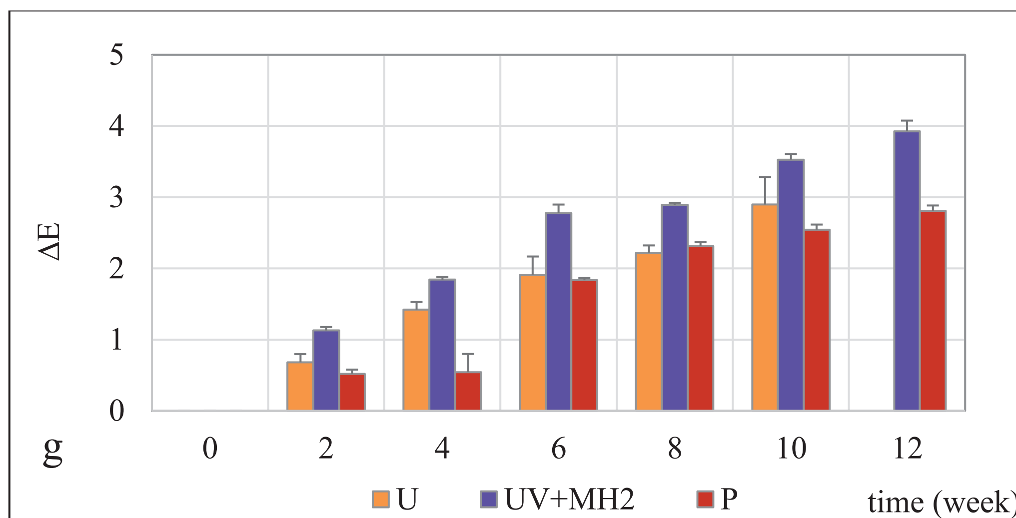


Figure 4.8. (cont.)

4.3. Conclusions

In this chapter, freshly squeezed verjuice was pasteurized by a combined UV-C irradiation and mild heat (UV+MH2) treatment. For this purpose, verjuice samples were subjected to 5D pasteurization considering the process conditions that were previously determined for UV+MH2 treatment in Chapter 3 (i.e., four UV lamps on configuration, six cycles, 3.80 mL/s flow rate, 372 s exposure time, 77.98±0.26 mJ/mL UV dose at 51.24±2.74 °C). Conventional thermal pasteurization (P) (72 °C for 18 s) and untreated (U) verjuice samples were also used as a positive and negative control samples during 12 weeks of refrigerated storage (4.22±1.15 °C). Microbiological (total mesophilic aerobic bacteria count, yeasts and molds and total coliforms), physicochemical and optical properties (pH, total soluble solids content, titratable acidity, absorption coefficient, turbidity, color parameters) were monitored during storage.

Untreated verjuice was completely spoiled by the growth of yeast and molds at the end of the 10 weeks of storage period. The YM count was higher than 3 logs (3.02±0.41 log CFU/mL) exceeding the microbiological criteria mandated by Turkish Food Codex. UV+MH2 treated and thermally pasteurized verjuice exhibited no microbial growth during 12 weeks of refrigerated storage.

Some of the physicochemical properties of verjuice samples, i.e. pH, total soluble solid content (TSS) and titratable acidity (TA) values, did not change during the entire 12 weeks of storage period. However, optical properties, i.e. absorption coefficient, turbidity and color parameters, changed during storage ($p \leq 0.05$). A slight increase was detected in

the absorption coefficient of UV+MH2 treated and P verjuice samples at the end of the 12th week of refrigerated storage. Turbidity values were significantly increased by storage time in untreated (U) and treated (UV+MH2 and P) verjuice samples. Considering color properties, L* (lightness-darkness) values decreased in untreated and treated verjuice samples. a*(redness-greenness) and b*(yellowness-blueness) values of untreated and treated verjuice samples varied significantly during storage. However, change of b* value in UV+MH2 treated verjuice (17%) was not pronounced as much as in the U (54%) and P (59%) verjuice samples. Hue angle of UV+MH2 treated verjuice did not predominantly change, i.e. it was around in the yellow color (90°) during 12 weeks. The chroma value of UV+MH2 treated verjuice was protected better than untreated and P treated verjuice. However, it was increased significantly in all samples during refrigerated storage, i.e. the color of all verjuice samples became more saturated with time. The increase of the browning index (BI) in all verjuice samples implied that the browning was developed during the storage period. The browning was less in the UV+MH2 treated verjuice compared to other samples. Total color change of the UV+MH2 treated verjuice (ΔE: 3.93) was well visible at the end of 12 weeks, it was in noticeable range in the U (ΔE: 2.90) and P (ΔE: 2.81) treated sample. In conclusion, UV-C irradiation at moderate temperatures (hurdle technology) which provided longer shelf life could be suggested as an alternative to thermal pasteurization of verjuice. On the other hand, the system parameters (flow rate, exposure time, path length etc.) required to be improved in future UV reactor desings in order to ensure minimal quality change.

CHAPTER 5

VERJUICE PASTEURIZATION BY PULSED-UV LIGHT (PUV) TREATMENT

Fruit juice is becoming a more popular drink with a consumption of 10.3 billion litersin Europe by 2017 (European Fruit Juice Association [EFJA], 2017). Nowadays, consumers demand high-quality and fresh-like food products (Koutchma et al., 2009). Outbreaks associated with fruit juice consumption are a burgeoning health concern. Thermal pasteurization is traditionally used to inactivate pathogens and spoilage microorganisms, as well as enzyme spoilers, in fruit juice. The treatment is principally performed at 65 °C for 30 min, 77 °C for 1 min and 88 °C for 15 s (Miller & Silva, 2012). However, this thermal process may lead to important chemical and physical changes that impair organoleptic properties, degrade nutrients and reduce the content of some bioactive compounds in juices (Gomez, Welti-Chanes, & Alzamora, 2011; Rawson et al., 2011; Tiwari et al., 2009a; Wang et al., 2018). In order to fulfil the demands of consumers and prevent the undesirable effects of thermal pasteurization, alternative non-thermal technologies, such as high hydrostatic pressure, pulsed electric field (PEF), ultrasound (US), ultraviolet C (UV-C) radiation, and high-intensity pulsed-ultraviolet light (PUV) have been developed (Paniagua-Martinez, Ramirez-Martinez, Serment-Moreno, Rodrigues, & Ozuna, 2018; Raso & Barbosa-Canovas, 2003). These non-thermal technologies can also be combined with mild heat treatment to enhance microbial inactivation (Ross et al., 2003).

PUV technology is used as a non-thermal processing method to eliminate pathogenic and spoilage microorganisms in foods. It is applied to solid and liquid foods by using short time (1 μs to 0.1 s) high-peak pulses between 200 and 1100 nm (Barbosa-Canovas et al., 1998; Oms-Oliu et al., 2010b). PUV can be described as a multi-target inactivation process with photochemical (cellular DNA damage), photothermal (overheating of the cell) and photophysical effects (cell membrane destruction) being involved (Gomez-Lopez et al., 2007; Krishnamurthy, Demirci, & Irudayaraj, 2007). Thus, microbial inactivation by PUV is not equivalent to continuous UV irradiation. Pulsed UV-C light (200-280 nm) has a great impact on the inactivation of microorganisms

(Gomez-Lopez et al., 2007; Koutchma, 2008). The United States Food and Drug Administration (U.S. FDA) has approved the use of PUV on food and food surfaces under strict conditions (U.S. FDA, 1996). Some critical factors affect the performance of PUV, including the distance from the lamp, sample thickness, contamination level of sample and sample composition (Gomez-Lopez et al., 2007). These variables should be considered to increase the inactivation efficacy of the PUV process. Studies about PUV indicate that this technology could be a promising alternative method for the decontamination of several solid foods, such as meat products (Ganan, Hierro, Hospital, Barroso, & Fernandez, 2013), vegetables (Gomez-Lopez, Devlieghere, Bonduelle, & Debevere, 2005) and fruits (Koh, Noranizan, Karim & Hanani, 2016; Ramos-Villarroel et al., 2011), as well as liquid foods, such as fruit juices (Ferrario, Alzamora, & Guerrero, 2013; Hwang, Cheigh, & Chung, 2015; Maftei et al. 2014; Pataro et al., 2011; Sauer & Moraru, 2009). PUV technology can also be combined with other technologies (US, thermosonication, PEF, mild heating, etc.) to increase its antimicrobial effect in foods (Caminiti et al., 2011b; Ferrario & Guerrero, 2016; Ferrario et al., 2015; Hilton et al., 2017; Munoz et al., 2011).

This chapter describes the pasteurization of verjuice (unripe grape juice) by using nonthermal PUV technology to extend its shelf life with a minimum quality loss. The study presented in this chapter was conducted in three main steps. In the first step, various processing parameters, such as the depth of juice layer, distance from the light and the number of pulses were examined, using a bench-top PUV system. The efficacy of the PUV system was determined, based on the logarithmic reduction of a target microorganism in verjuice, i.e., *Saccharomyces cerevisiae* (NRRL Y-139). The conditions providing maximum reduction of *S. cerevisiae* were selected after all experiments. In the second step, the selected PUV treatment conditions were combined with mild heating (PUV+MH) at several different temperatures to achieve the 5-log reduction (5D) of *S. cerevisiae* cells. In the third or last part of this chapter, several quality properties of verjuice pasteurized with the combined PUV+MH treatment were monitored during the shelf life. The quality properties were compared with commercially thermal-pasteurized (P) and untreated (U) verjuice samples, as positive and negative controls, respectively.

Experiments covered in this chapter were performed in the laboratories of the Novel Technologies for Food Processing Research Group, Department of Food Technology, University of Lleida (UDL), Spain.

5.1. Materials and Methods

5.1.1. Raw Materials: Unripe Grapes and Verjuice Preparation

Unripe grape berries and freshly-squeezed verjuice, i.e., raw materials for the shelf life and all microbial inactivation studies, were prepared according to the procedures detailed in sections 3.1.1 and 3.1.2 of Chapter 3. Unripe grape berries were placed in plastic pouches (500 g), vacuum-packaged and then frozen. Freshly-squeezed verjuice was thermally pasteurized (72 °C/18 s), placed in sterile plastic bottles (50 mL) and frozen. Frozen raw materials (grape pouches and verjuice bottles) were transported to the University of Lleida in Spain, within ice bags and stored frozen (-20 °C) until use.

Verjuice bottles were used for all microbial inactivation experiments after defrosting. Unripe grape berry pouches were defrosted and squeezed by a fruit juice extractor. Detartarisation was applied by keeping the juice refrigerated (4 °C) for 1 day, to precipitate tartrate particles. Clear verjuice was obtained after filtering with a sterile double-layer cheesecloth and used for the shelf life study.

5.1.2. Verjuice Characterization

Physicochemical properties of verjuice, i.e., pH, total soluble solids (TSS, °Brix), titratable acidity (TA, %), and optical properties, i.e., absorption coefficient (cm^{-1}), turbidity (NTU), color parameters (*L** [lightness], *a** [redness/greenness], *b** [yellowness/blueness], chroma [*C**], hue angle [*h*°], total color change [ΔE], browning index [BI]), were determined before PUV treatment. All analyses were performed as described in Chapter 4 (section 4.1.5), using the lab facilities of Lleida University. The pH, TSS content (ºBrix), absorption coefficient (cm^{-1}) and turbidity (NTU) of verjuice were measured using a bench-top pH meter (Crison Instruments SA, Barcelona, Spain), a hand-held refractometer (Palette PR-32, Atago USA, Inc.), a spectrophotometer (Jasco V-650, Jasco Europe Srl, Italy) and a portable turbidimeter (HI 98703, Hanna Instruments, Inc., USA), respectively. The CIE *L**, *a**and *b** color parameters were measured directly, using a Minolta CR-400 chromometer (Konica, Inc., Japan), and *h*° *C** and ΔE then calculated by equations given in section 4.1.5.6.

5.1.2.1. Antioxidant Activity of Verjuice

The antioxidant activity of verjuice samples was investigated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method of Miliauskas, Venskutonis, and Van Beek (2004) and Pataro, Sinik, Capitoli, Donsi, and Ferrari (2015), with slight modifications. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. The antioxidant effect is proportional to the disappearance of DPPH in test samples. DPPH shows a strong absorption maximum at 517 nm (purple), which forms a yellow solution upon abstraction of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms abstracted. Therefore, the antioxidant capacity can be easily evaluated by monitoring the decrease of UV absorption at 515–517 nm. The greater the DPPH radical consumption, the higher the antioxidant activity of the sample.

The DPPH radical solution was prepared daily by dissolving in methanol (25 ppm). A reagent solution consisting of 3.9 mL DPPH solution, 10 μL verjuice sample and 90 μL deionized water were placed in a plastic cuvette (1 cm diameter) and the mixture was kept in the dark at room temperatures (20 °C) for 60 min. The blank sample was composed of 3.9 mL DPPH solution and 100 μL deionised water instead of verjuice. Autozero was done with methanol. After 60 min, the absorbance values were measured at 515 nm. Each sample was prepared in triplicate. The antioxidant activity or radical scavenging activity of verjuice based on DPPH inhibition (%) was calculated from Equation 5.1, where the absorbance of the sample (after 60 min) and blank were identified as A_s and A_b, respectively.

$$DPPH\ inhibition\ (\%) = 100 - (\frac{A_s}{A_b} x100\ ) \quad (5.1)$$

5.1.3. Microbiological Analysis: Background Microflora, Target Microorganism, Sample Inoculation and Enumeration

No background microflora (total aerobic mesophilic bacteria count [TAMC], yeasts and mould count [YMC] and total coliforms [TC]) were detected in verjuice

samples before the microbiological inactivation studies, by applying the growing mediums and incubation conditions presented in section 3.1.4.1.

Based on the natural fermentation results (section 3.1.4.2), it was decided to use *S. cerevisiae* (NRRL Y-139) yeast strain as a target microorganism for verjuice. Stock cultures of *S. cerevisiae* (NRRL Y-139) containing 25% glycerol were transferred to Lleida University and stored (-80 °C) before use. *S. cerevisiae* was cultured in yeast extract-peptone-dextrose (YPD) broth (manually prepared from 10 g yeast extract, 20 g peptone and 20 g dextrose per 1 L distilled water). *S. cerevisiae* colonies were spread on potato dextrose agar (PDA; Merck, Darmstadt, Germany) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) and enumerated after incubation at 30 °C for 48 h.

The *S. cerevisiae* strain was adapted to high acidic conditions before inoculation into verjuice, by reducing the pH of the YPD broth. Firstly, 100 µL of the stock culture of *S. cerevisiae* (NRRL Y-139) was inoculated to 100 mL YPD broth (pH 3.5) and incubated in a shaker at 200 rpm and 30 °C for 24 h. After incubation, 1 mL of enriched cells in YPD broth (pH 3.5) was transferred to the YPD broth at pH 2.7, which was close to the pH of the verjuice, and incubated in the shaker for 48 h under the same conditions (30 °C, 200 rpm). The cell concentration reached approximately 10^6 CFU/mL. The acid-adapted yeast cells were inoculated to verjuice and enumerated at several different time intervals during incubation (30 °C, 200 rpm). The colonies were counted on the PDA acidified to pH 3.5 with 10% tartaric acid. It was observed that the number of viable yeast cells did not significantly change in the highly acidic conditions of verjuice during incubation for 6 h, during which time, the acid-adapted viable *S. cerevisiae* cell count was in the range of 6.1-5.9 log CFU/mL (Figure 5.1). Finally, the acid-adapted *S. cerevisiae* (NRRL Y-139) cells were collected and plated on the PDA slants (acidified to pH 3.5 with 10% tartaric acid), incubated at 30 °C for 2 days and then stored (4 °C) before use in the PUV, MH and PUV+MH microbial inactivation experiments.

5.1.4. Experimental Procedure of Microbial Inactivation Study

Figure 5.2 outlines all the microbial inactivation studies conducted in this chapter. Initially, frozen verjuice bottles were defrosted and inoculated with acid-adapted *S. cerevisiae* (NRRL Y-139) cells.

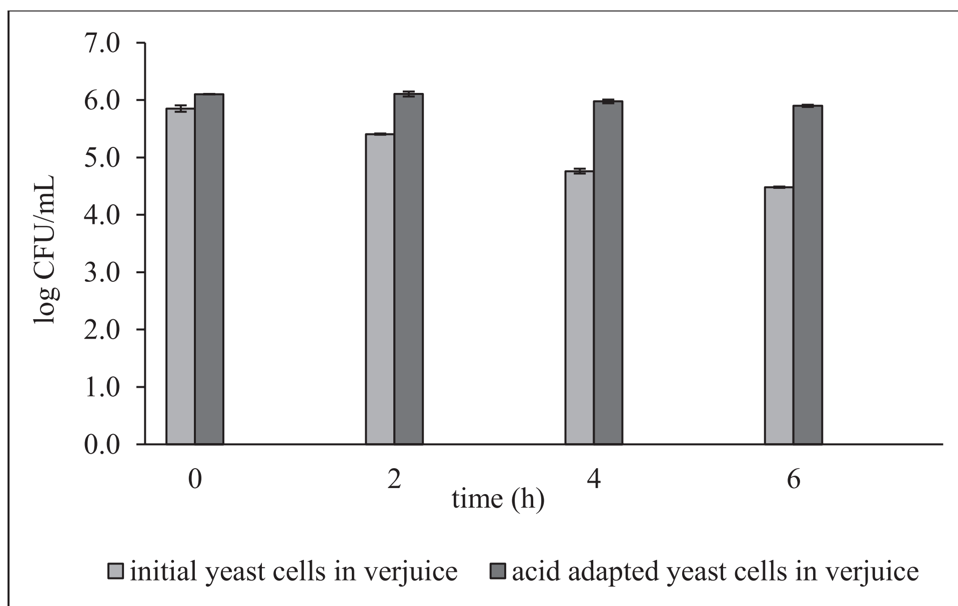


Figure 5.1. The viability of *S. cerevisiae* (NRRL Y-139) in verjuice before and after acid adaptation (light grey: initial cells, dark grey: acid adapted cells)

The initial microbial load of verjuice was adjusted to ~10^5 CFU/mL (5 log CFU/mL). Inoculated verjuice was exposed to PUV, MH and the combined PUV+MH treatments, respectively. All treatments were conducted by using a bench-top PUV system, i.e., PUV treatment was applied by switching xenon lamps on at room temperature, MH treatment used the same system but the xenon lamps were switched off, and combined PUV+MH treatment was performed by switching the xenon lamps on at mild temperatures. The PUV+MH treatments applied to verjuice combined the most effective PUV treatment conditions, i.e., the conditions that achieved the highest logarithmic inactivation of *S. cerevisiae* in verjuice, with three different mild temperatures. The effects of all treatments in verjuice were then evaluated based on the 5D reduction of the target microorganism (i.e., *S. cerevisiae* NRRL Y-139), for verjuice pasteurization.

All of the microbial inactivation experiments in this section were necessary for the next shelf life study (section 5.1.5) in which verjuice was pasteurized by the best PUV processing conditions. The verjuice samples were then stored at refrigerated (4 °C) and room temperature (25 °C) conditions for 12 weeks. The positive (P) and negative (U) controls (refer to the introduction) were used to compare the quality parameters of the verjuice samples during its shelf life.

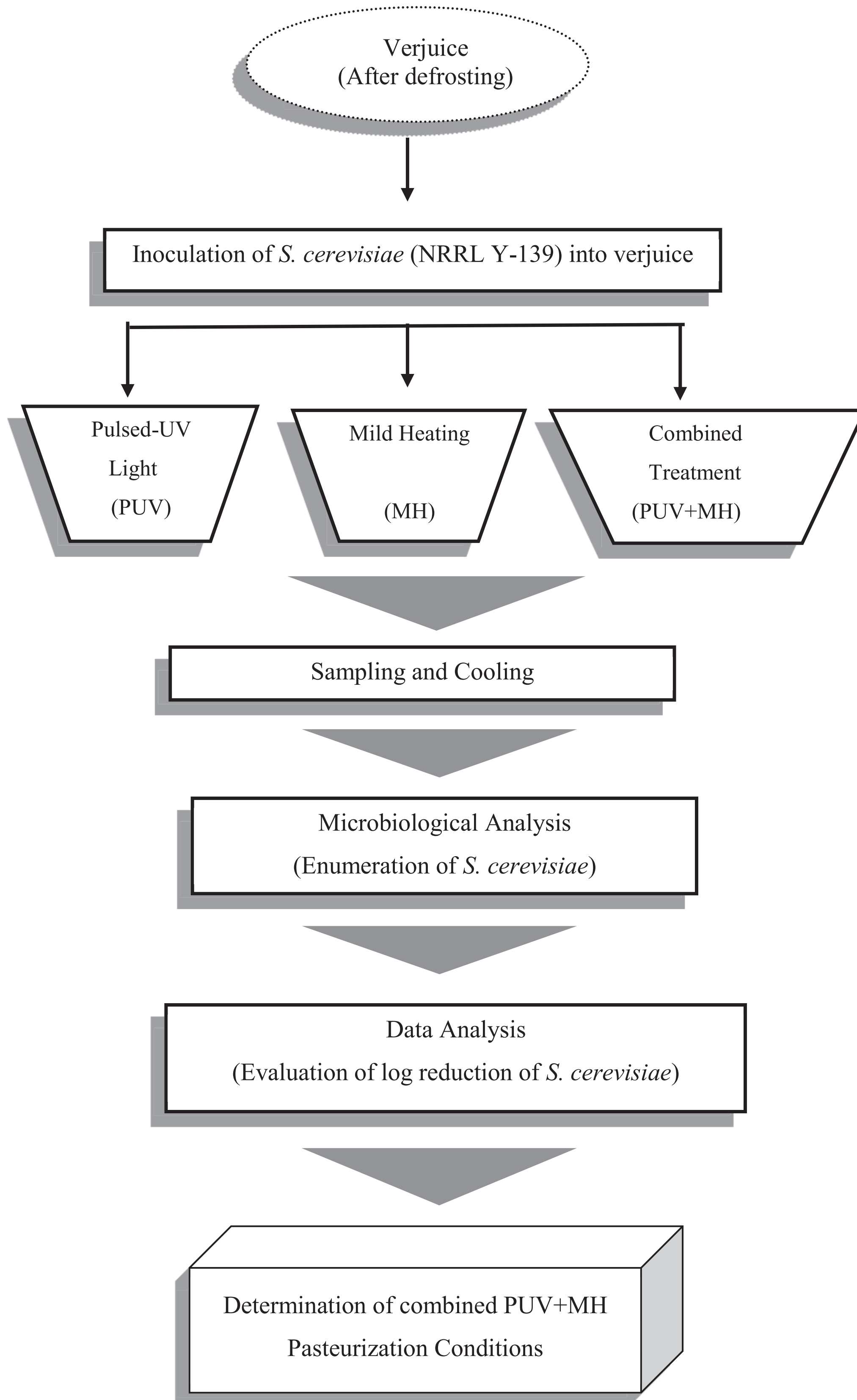


Figure 5.2. The flow diagram of microbial inactivation studies conducted in Chapter 5

5.1.4.1. Pulsed-UV Light (PUV) Treatment of Verjuice

Verjuice samples inoculated with acid-adapted *S. cerevisiae* (NRRL Y-139) strains were exposed to PUV by using an automatic laboratory-scale bench-top XeMaticA-2L System (SteriBeam Systems GmbH, Germany; Figure 5.3). The device consists of two standard air-cooled UV-C transparent quartz xenon lamps (18-cm long, inner and external diameters of 1 and 9 mm, respectively) located above and below the device, a stainless-steel table to place the samples in the instrument, a chamber door to close the system, and power and control panels. The emitted spectrum in the system ranged from 180 to 1100 nm, with 15-20% of the light in the UV region. Each pulse lasted 0.3 ms. The sample received 0.68 and 0.34 J/cm^2 per pulse when placed at 5 and 10 cm from the lamp located above the sample table, respectively.

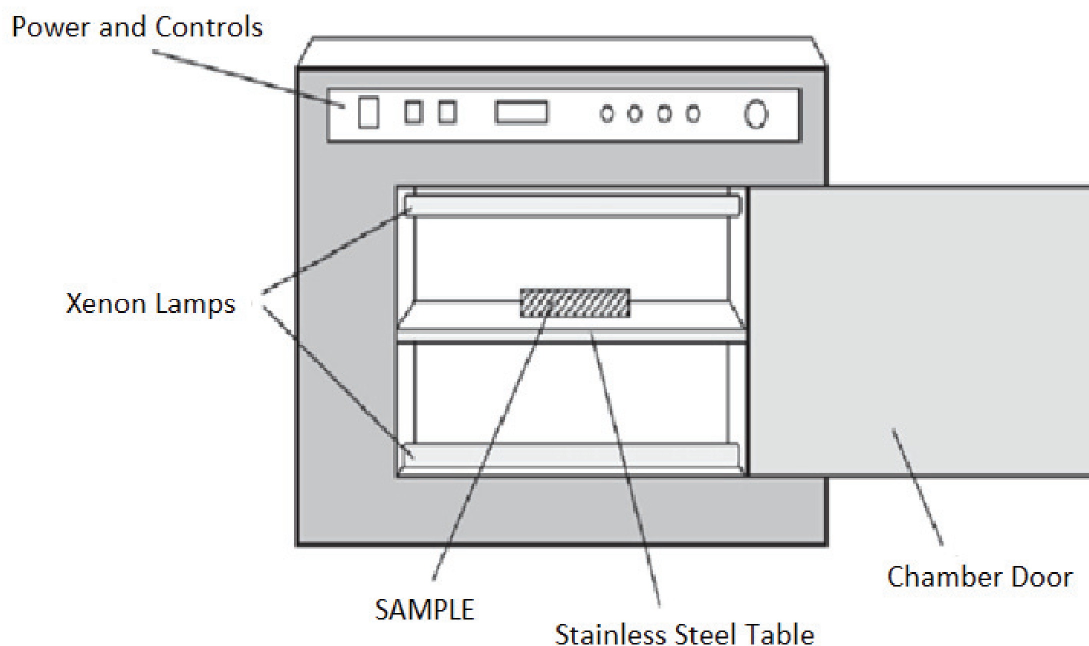


Figure 5.3. Schematic view of Laboratory Scale Bench Top Pulse Light System

In order to evaluate the conditions of PUV treatment on *S. cerevisiae* (NRRL Y-139) inoculated into verjuice, the effects of juice layer depth (1, 3, 5 mm), distance of the juice from the xenon lamp (5 and 10 cm) and number of pulses (0, 10, 20, 30, 40 and 50) were investigated using the bench-top PUV equipment. The experiments were coded as PUV5-1, PUV5-3 and PUV5-5 for 1, 3 and 5 mm depth of verjuice placed at 5 cm

distance; and PUV10-1, PUV10-3 and PUV10-5 for 1, 3 and 5 mm depth of verjuice placed at 10 cm distance from the lamp, respectively. Table 5.1 lists the design of all the PUV experiments. Verjuice was poured into a small petri dish (5 cm diameter) and inoculated with acid-adapted *S. cerevisiae* (NRRL Y-139) before PUV treatment. Different depths of verjuice layer having an initial load of ~10^4-10^5 CFU/mL *S. cerevisiae* were placed at 5 and 10 cm distance from the xenon lamp. The samples were exposed to up to 50 pulses at 0.68 and 0.34 J/cm^2 per pulse for each distance, respectively, for a maximum 20 min. Each treatment was implemented with 10 pulses per 4 min to prevent overheating the PUV system. The overall PUV dose after treatments was calculated as ranging from 0 to 34 J/cm^2 (for the 5 cm distance) and from 0 to 17 J/cm^2 (for the 10 cm distance). Verjuice was cooled within 10 s, by opening the door of the PUV system after every 10 pulses. Thus, the temperature was controlled, and lethal heating did not occur during the processes (under 35 °C). All experiments listed in Table 5.1 were repeated three times.

Table 5.1 PUV treatment conditions used in this chapter

Experiment	Treatment code	Juice layer depth (mm)	Distance from the lamp (cm)	Number of pulses
1	PUV5-1	1	5	0, 10, 20, 30, 40, 50
2	PUV10-1	1	10	0, 10, 20, 30, 40, 50
3	PUV5-3	3	5	0, 10, 20, 30, 40, 50
4	PUV10-3	3	10	0, 10, 20, 30, 40, 50
5	PUV5-5	5	5	0, 10, 20, 30, 40, 50
6	PUV10-5	5	10	0, 10, 20, 30, 40, 50

5.1.4.2. Mild Heating (MH)

Hilton et al. (2017) demonstrated that PUV treatment was not very efficient for decontamination of liquids under sub-lethal temperatures but obtained synergistic effect between PUV and temperatures above 40 °C for the inactivation of *Listeria innocua* in a phosphate buffer solution. It is known that yeasts are more resistant to PUV than bacteria (Hilton et al., 2017; Tran & Farid, 2004). For these reasons, mild temperatures above 40

°C were selected in this study. Three different MH temperatures (43, 45, 47 °C) were applied to verjuice inoculated with 10^4-10^5 CFU/mL acid-adapted *S. cerevisiae* (NRRL Y-139) using the PUV system with the xenon lamps switched off. Verjuice samples were poured into a glass-jacketed beaker, and the beaker was connected to a temperature-controlled water bath. The beaker was placed inside the PUV equipment (lamps off) and the internal temperature of verjuice in the beaker was adjusted to 43, 45 and 47 °C, controlled using a K-type thermocouple. Next, verjuice inoculated with *S. cerevisiae* (NRRL Y-139) was exposed to MH treatments, i.e., 43 °C for 20 min (MH43), 45 °C for 20 min (MH45) and 47 °C for 8.5 min (MH47), respectively. The reason for choosing less time at 47 °C was that all yeast cells in verjuice began to die after 8.5 min at 47 °C and no cell count was detected after that time. Samples taken at specific intervals during the MH treatments were rapidly cooled in an ice bath. The efficacy of MH treatment alone on the inactivation of *S. cerevisiae* in verjuice was then evaluated by the enumeration of the *S. cerevisiae* colonies in the verjuice samples before and after the MH treatments by spread plating on PDA. Table 5.2 provides the MH conditions used in the study. All experiments in Table 5.2 were carried out in triplicate.

Table 5.2. MH and PUV+MH treatment conditions used in the chapter

Experiment	**Treatment code**	**Juice layer depth (mm)**	**Temperature (°C)**	**Number of pulses**
1	MH43	3	43	Not applicable (N/A)
1	PUV+MH43-3	3	43	0, 10, 20, 30, 40, 50
2	MH45	3	45	N/A
2	PUV+MH45-3	3	45	0, 5, 10, 15, 20, 30, 40, 50
3	MH47	3	47	N/A
3	PUV+MH47-3	3	47	0, 3, 6, 9, 12, 15, 18
4	MH43	5	43	N/A
4	PUV+MH43-5	5	43	0, 10, 20, 30, 40, 50
5	MH45	5	45	N/A
5	PUV+MH45-5	5	45	0, 5, 10, 15, 20, 30, 40, 50
6	MH47	5	47	N/A
6	PUV+MH47-5	5	47	0, 3, 6, 9, 12, 15, 18

5.1.4.3. Pulsed-UV Light Assisted with Mild Heating (PUV+MH)

Besides the individual applications of PUV and MH treatments, the combined PUV+MH was studied as a hurdle technology to increase the logarithmic reduction of *S. cerevisiae* (NRRL Y-139) and to achieve 5D reduction of a target microorganism in verjuice. Thus, the effect of "hurdle treatment" on the microbial quality of verjuice was also evaluated.

Combined PUV+MH experiments were conducted under the same conditions as the MH treatments but with the xenon lamps turned on. Verjuice was poured into the jacketed beaker used in MH treatment, placed into the PUV equipment (xenon lamps on) at a level to produce two different depths (3 and 5 mm) and connected to the water bath (1 mm depth of juice layer was not studied due to the evaporation problems occurred during mild heat conditions). The internal temperature of the verjuice was adjusted to 43, 45 and 47 °C, controlled using the K-type thermocouple. Next, the verjuice inoculated with *S. cerevisiae* (NRRL Y-139) was exposed to simultaneous PUV and MH treatment at a distance of 10 cm from the lamp, by applying the maximum number of pulses used in the PUV treatments given in Table 5.1, i.e. 50 pulses at 43 °C (20 min), 50 pulses at 45 °C (20 min) and 18 pulses at 47 °C (8.5 min). The number of the pulses in the PUV+MH treatments was arranged based on the total exposure time of MH treatment alone. Table 5.2 gives the process parameters of the six combined treatments, coded as PUV+MH43-3, PUV+MH45-3, PUV+MH47-3, PUV+MH43-5, PUV+MH45-5, and PUV+MH47-5. Samples were removed at specific pulse intervals during PUV+MH treatments and immediately cooled in an ice bath. The number of *S. cerevisiae* colonies in the verjuice samples were counted by spread plating on PDA. All combined PUV+MH experiments in Table 5.2 were repeated three times.

5.1.4.4. Dose Calculation of Pulsed-UV Light (PUV)

The dose of PUV applied to verjuice was determined based on the fluence of the pulse measured at the desired distance from the xenon lamp in the bench-top PUV system. The principle of the PUV dose measurement was explained in Llano, Marselles-Fontanet, Martin-Belloso, and Soliva-Fortuny (2016). First, the energy accumulated by each pulse (0.3 ms) was measured by using a photodiode detector in the stainless-steel table located

at different distances (from 4 to 14 cm) from the xenon lamp (located above the sample table) in the system. An oscilloscope was connected to the photodetector and the obtained signals converted to radiance values by using a standard light source as a calibration, according to the manufacturer's instructions. A plot of the fluence versus distance from the lamp was generated previously in the same system at Lleida University (Figure B.1 in Appendix B). The exponential equation of this graph (Figure B.1), i.e. $y=3.3743x^{-1.004}$, where x is the distance (cm), and y is the fluence or PUV dose (J/cm^2), was used to calculate the corresponding doses in this chapter. Thus, PUV doses were calculated as 0.34 and 0.68 J/cm^2/pulse for 10 and 5 cm distance from the xenon lamp in the system, respectively. The total PUV dose (J/cm^2) applied to verjuice was determined by multiplying the number of the pulses of each treatment by the dose calculated per pulse.

5.1.5. Shelf Life Study

Freshly-squeezed verjuice was used for the shelf life study. Vacuum-packed and frozen unripe grapes were thawed and squeezed by a juice extractor, and a detartarisation step was applied to cloudy juice by refrigeration (4 °C) for 24 h, followed by filtration through cheesecloths. The clear verjuice obtained was pasteurized by the PUV+MH condition that achieved a 5D reduction of *S. cerevisiae*. The shelf life of the positive (P) and negative (U) controls were also examined in the study. Microbiological, physicochemical, optical and antioxidant activity properties of untreated and treated verjuice were evaluated during the shelf life.

5.1.5.1. Thermal Pasteurization of Verjuice

Conventional thermal pasteurization conditions of verjuice were determined as 72 °C for 18 s (section 4.1.1), by calculating the thermal inactivation kinetic parameters, i.e., D- and z-values of *S. cerevisiae* (NRRL Y-139) in verjuice. Freshly-squeezed verjuice was processed by using a continuous heat pasteurization system, composed of a stainless-steel capillary tube, pump, thermostatic bath (Huber GmbH, Offenburg, Germany) and a cooling cabinet (adjusted to 4 °C), as demonstrated in Figure 5.4. Freshly-squeezed verjuice (900 mL) was poured into a 1 L sterile glass bottle and pumped at 1.02 mL/s for 18 s through the capillary tube (72 °C) located in the thermostatic bath. After the

pasteurization step, the juice in the capillary tube was immediately pumped to the cooling cabinet. The cooled and pasteurized verjuice was collected into a 1 L sterile glass bottle and ready to use for the shelf life study.

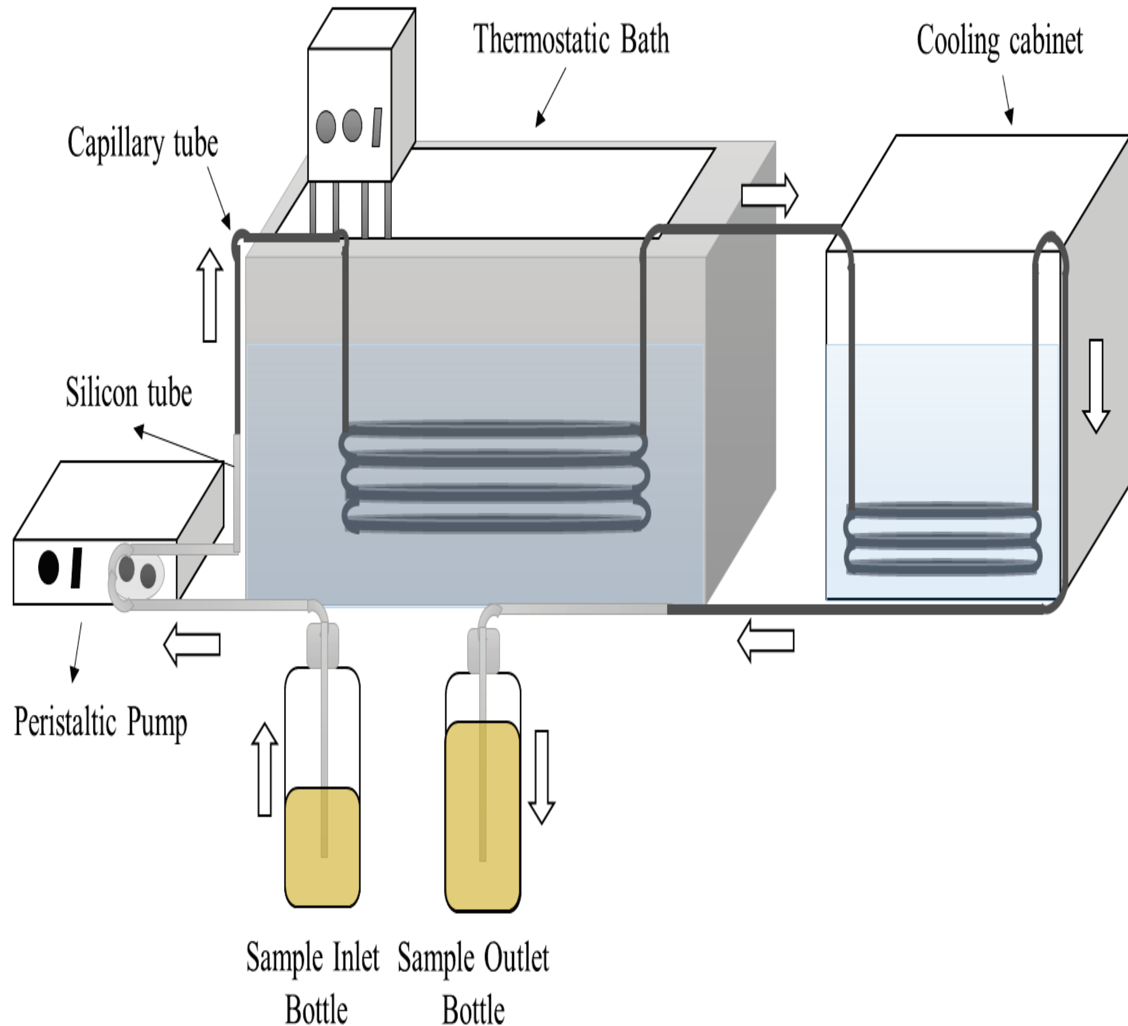


Figure 5.4. Continuous Heat Pasteurization System used in Chapter 5

5.1.5.2. Combined Pulsed-UV Light and Mild Heating (PUV+MH) Pasteurization of Verjuice

Freshly-squeezed verjuice was pasteurized under the selected combined PUV+MH47 process conditions that achieved a 5D reduction of *S. cerevisiae*, i.e., 6.12 J/cm^2 total PUV dose, 3 mm deep juice layer, 10 cm distance from the lamp, 47 °C and total exposure time of 8.5 min.

For the combined PUV+MH47 pasteurization treatment, freshly-squeezed verjuice was poured into the jacketed beaker until reaching a 3 mm juice depth (6.05 mL) and placed at 10 cm distance from the lamp within the bench-top PUV instrument. The beaker was connected to the water bath, and the internal temperature of verjuice was adjusted to 47 °C, controlled using a K-type thermocouple. Simultaneous PUV+MH treatment was done by exposing verjuice to up to 18 pulses of PUV at 47 °C. The treatment was completed within 8.5 min. After each combined PUV+MH47 treatment, pasteurized verjuice sample (6.05 mL) was collected in a sterile amber glass bottle and cooled (4 °C) until sufficient volume (1 L) was obtained for the shelf life study.

5.1.5.3. Storage and Analyses of Verjuice during its Shelf Life

Approximately 25-30 mL of the controls (U and P [72 °C for 18 s]) and combined PUV+MH (PUV+MH47) pasteurized verjuice were transferred to sterile dark glass bottles (50 mL) and stored under refrigerated (4 °C) and room temperature (25 °C) conditions for 6 weeks, with periodic sampling at 0, 1, 2, 4 and 6 weeks. The experiments were done in triplicates. The storage temperature was checked during the shelf life at specific intervals and remained constant (25 °C). The refrigeration temperature was around 4.94±0.89 °C. Figure 5.5 depicts the steps involved in the shelf life study.

All the samples obtained were analysed for microbiological (TAMC, YMC, TC), physicochemical (pH, TSS content, TA), optical (absorption coefficient, turbidity, color) and the DPPH antioxidant attributes, according to the procedures and equipment detailed in sections 5.1.2 and 5.1.3.

5.1.6. Data Analyses

All microbial inactivation and shelf life study experiments were repeated three times. The results, given as the logarithmic reductions of acid-adapted *S. cerevisiae* (NRRL Y-139) in verjuice obtained from the different treatment conditions, as well as the quality properties of verjuice during its shelf life, were expressed as their means and standard deviations. Regression analysis was applied by using a commercial spreadsheet (Microsoft Excel, Redmond, WA, USA).

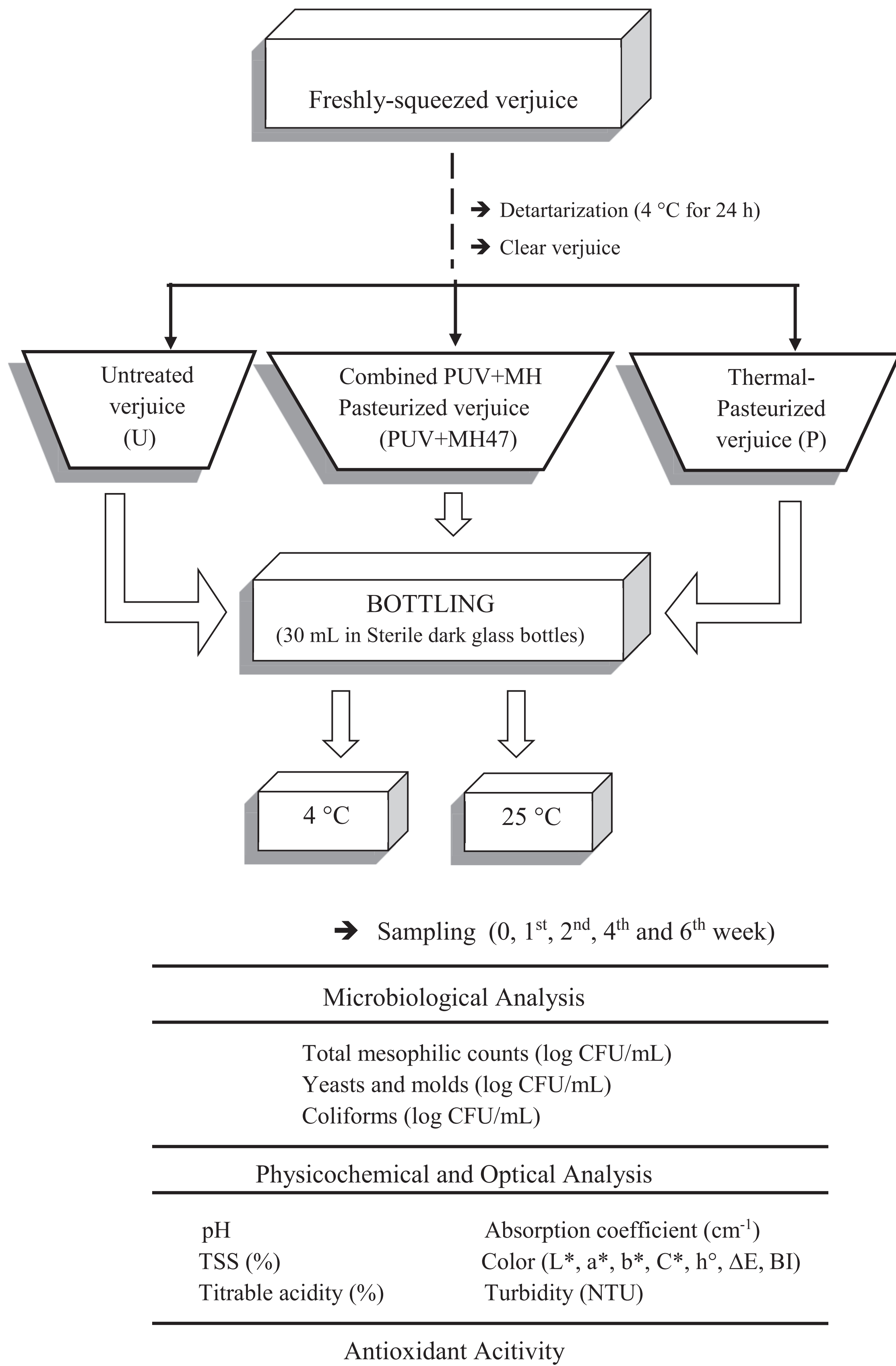


Figure 5.5. Diagram of the shelf life study of freshly squeezed verjuice

One-way analysis of variance (ANOVA) was done to determine how significantly the independent variables (U, P and PUV+MH treatments) affected the changes in the dependent variables (microbiological, physicochemical, optical, DPPH antioxidant capacity properties) during the shelf life of verjuice. A least significant difference (LSD) test was applied, and the means of data were compared by Tukey's pairwise comparison test at a 95% confidence interval. The level of significance was set at $p \leq 0.05$. Minitab 16 software program (Minitab, Inc., State College, PA, USA) was used for the one-way ANOVA and LSD test of the results.

5.2. Results and Discussion

5.2.1. Characterization of Verjuice

Table 5.3 reveals the physicochemical and optical properties of defrosted verjuice (pre-pasteurized and frozen before transportation to Lleida University), i.e., pH, TSS (°Brix), TA (%), absorption coefficient (cm^{-1}), turbidity (NTU) and CIE color parameters (*L**, *a**, *b**). All properties of verjuice used in this chapter were slightly acidic, more transparent, marginally darker and yellower (higher *L** and *b** values) compared to the freshly-squeezed verjuice used in Chapters 3 and 4 (Table 3.1). The reason for this difference could be the pasteurization treatment applied to verjuice before it was frozen. According to the physicochemical properties, verjuice is a highly acidic juice due to its pH (2.58) and TA content (1.99%). The TSS content of verjuice made from grapes harvested before ripening was measured to be much lower, i.e., 3.70 °Brix, than mature grape juice, which has a minimum 15 °Brix content, according to Codex Standards (Alimentarius, 1981b). These properties of verjuice were similar to the verjuice used by Hayoglu et al. (2009) (pH 2.91, TA 3.00%, TSS 4.5 °Brix) but with slight differences, possibly attributed to the different cultivation conditions of grapes (Bates et al., 2001; Somogyi et al., 1996). The turbidity (27.20 NTU) and absorption coefficient (19.79 cm^{-1}) values of the cloudy and opaque verjuice contrasted with the properties of commercial grape juice (turbidity: 0.87 NTU; absorption coefficient: 5.49 cm^{-1}) used in work by Kaya and Unluturk (2016). The CIE *L* a* b** color parameters of verjuice were 35.24, 0.26 and 4.36, respectively. The yellow was noticeable in verjuice due to the presence of carotenoid pigments in its composition (Lancaster et al., 1997).

Table 5.3. Physicochemical and optical properties of verjuice

pH		2.58	±0.01
Total soluble solids (°Brix)		3.70	±0.00
Titratable acidity (%)		1.99	±0.03
Turbidity (NTU)		27.20	±0.71
Absorption coeffient (cm^{-1})		19.79	±0.00
Color	**L***	35.24	±0.44
	a*	0.26	±0.09
	b*	4.36	±0.27

Results were presented as a mean±standard error (n = 3). TSS: total soluble content.

5.2.2. The Effect of Pulsed-UV Light (PUV) on *S. cerevisiae* Inoculated into Verjuice

Figure 5.6 (a and b) and Table 5.4 summarise the impact of PUV technology on the inactivation of acid-adapted *S. cerevisiae* (NRRL Y-139) strains in verjuice of different depths (1, 3, 5 mm) and distances from the xenon lamp (5, 10 cm). The results indicated that *S. cerevisiae* cells inoculated to verjuice were not efficiently inactivated by PUV alone, in all conditions. The highest reduction was 0.96±0.27 log CFU/mL for PUV5-1, i.e., the verjuice having a 1 mm depth exposed to PUV at a 5 cm distance, exposed to 50 pulses for 20 min (Figure 5.6a), which corresponded to a total PUV dose calculation of 34 J/cm^2 (Table 5.4).

According to Table 5.4, there was no statistical difference between the number of *S. cerevisiae* cells in verjuice having 3 mm (PUV5-3, 0.35±0.03 log CFU/mL reduction) and 5 mm depth (PUV5-5, 0.52±0.01 log CFU/mL reduction) exposed to 34 J/cm^2 PUV at 5 cm distance. However, the number of the cells were significantly reduced in the 1 mm verjuice depth (PUV5-1, 0.96±0.27 log CFU/mL reduction) exposed to the same dose of PUV, i.e. 34 J/cm^2, at the same distance ($p \leq 0.05$). Similarly, there was a significant difference between the *S. cerevisiae* count in verjuice having 1 and 5 mm depth exposed to PUV at 10 cm distance ($p \leq 0.05$). Therefore, the depth of juice layer was an important factor in this PUV system. It has been reported that 90% of the light can pass through only the first 1 mm layer of the juices (Keyser et al., 2008; Sizer & Balasubramaniam, 1999). The distance from the lamp could also be an important parameter, depending on the depth of verjuice. The efficacy of the PUV treatment was slightly lowered when the

distance between the verjuice and the lamp was increased from 5 to 10 cm. At 10 cm distance from the lamp (Table 5.4 and Figure 5.6b), *S. cerevisiae* cells were reduced to 0.60±0.03, 0.45±0.04 and 0.28±0.05 log CFU/mL after PUV10-1, PUV10-3, PUV10-5, respectively, at 17 J/cm^2. In the verjuice having 1 mm depth, the reduction of *S. cerevisiae* at a distance of 5 cm (PUV5-1) was significantly greater compared to 10 cm distance (PUV10-1) ($p \leq 0.05$). However, the distance was not a key parameter in the inactivation efficacy of the PUV system when using 3 and 5 mm depths of verjuice. The reason for the reduced efficacy of the PUV system could be the less dose calculated for the 10 cm distance (17 J/cm^2) relative to the 5 cm distance (34 J/cm^2) from the xenon lamp. Artiguez, Lasagabaster, and de Maranon (2011), and Said, Federighi, Bakhrouf, and Orange (2010) indicated that a high microbial inactivation in the liquids could be achieved with a high PUV dose, obtained by reducing the sample depth and the lamp distance and increasing the number of the pulses.

Maftei et al. (2014) studied the inactivation of *Penicillium expansum* moulds in clear apple juice under various PUV processing conditions and, similarly, the microbial reduction was improved (3.76 log CFU/mL decrease) in the juice with a low thickness (8 mm depth) and exposed to a high PUV dose (32 J/cm^2). The higher microbial reduction achieved in that study compared to the current investigation might be because the clarified apple juice absorbed PUV more efficiently than verjuice (obtained without the clarification step), even though the apple juice had more depth. In the PUV experiments by Ferrario et al. (2013), the inactivation of *S. cerevisiae* in natural apple juice (<1 log CFU/mL) was significantly lower than in the clarified juice (~4 log CFU/mL) after a PUV dose of 71.6 J/cm^2. It was also mentioned that the absorptivity of the light depends on the liquid media properties, such as suspended particles, color and TSS (Aguirre, Hierro, Fernandez, & de Fernando, 2014; Keyser et al., 2008).

Conversely, *S. cerevisiae* cells are highly resistant to PUV, due to having a larger size and different DNA structure than bacteria (Fredericks, du Toit, & Krugel, 2011; Tran & Farid, 2004). The PUV resistivity of *S. cerevisiae* in this study could be a result of using acid-adapted cells in verjuice, which affects the physiology of the cells, by altering the protein expression and membrane lipid composition (Huang, Tsai, & Pan, 2007; Yuk & Marshall, 2004), potentially alleviating the PUV effecting the DNA.

Consequently, PUV should be combined with another alternative method, such as MH, to increase its inactivation of *S. cerevisiae* in verjuice.

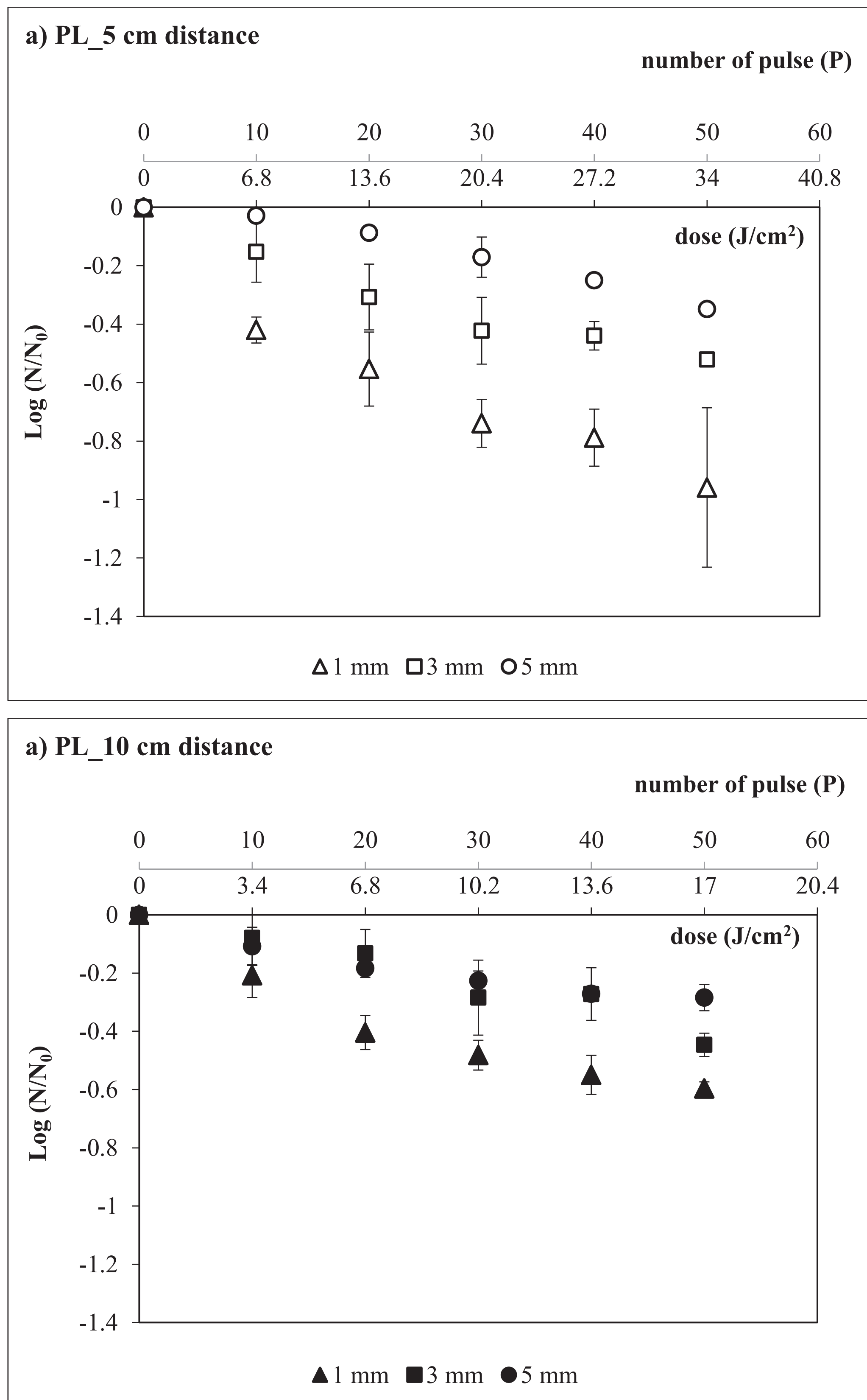


Figure 5.6. The inactivation of acid-adapted *S.cerevisiae* (NRRL Y-139) in verjuice by PUV treatment at different lamp distance 5 cm (a) and 10 cm (b) (juice depth Δ: 1mm, □:3mm, O:5mm)

Table 5.4. The log reductions of acid-adapted *S.cerevisiae* (NRRL Y-139) inoculated into verjuice by PUV treatment at different lamp distances and juice depth

Treatment code	Lamp distance (cm)	Depth of juice Layer (mm)	Log reduction (log No/N)	PUV dose (J/cm^2)
PUV5-1	5 cm	1 mm	0.96 ±0.27a	34
PUV5-3	5 cm	3 mm	0.52 ±0.01bc	34
PUV5-5	5 cm	5 mm	0.35 ±0.03bc	34
PUV10-1	10 cm	1 mm	0.60 ±0.03b	17
PUV10-3	10 cm	3 mm	0.45 ±0.04bc	17
PUV10-5	10 cm	5 mm	0.28 ±0.05c	17

Results were presented as "means± standard error". The least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different (p≤0.05).

5.2.3. The Effect of Mild Heating (MH) on *S. cerevisiae* Inoculated into Verjuice

The inactivation of acid-adapted *S. cerevisiae* (NRRL Y-139) strain in verjuice by using MH was evaluated at 43 °C/20 min (MH43), 45 °C/20 min (MH45) and 47 °C/8.5 min (MH47) (Figure 5.7). The maximum reduction of the acid-adapted *S. cerevisiae* cells was 3.4±0.44 log CFU/mL after 8.5 min of MH47 treatment, in comparison to 2.28±0.36 and 1.35±0.29 log CFU/mL after 20 min of MH45 and MH43 treatments, respectively. Thus, the high inactivation for MH47 was due to the lethal effect of heat on the yeast cells. In Figure 5.7, all MH inactivation curves showed upward concavity with a tailing phase, i.e., the reduction of *S. cerevisiae* cells in verjuice was significantly rapid within the first minutes of MH but then slowed down. Carrillo, Ferrario, and Guerrero (2018) observed the same trend for *S. cerevisiae* cells in a carrot–orange juice blend after MH at 50 °C and suggested the tailing resulted from the presence of heat-resistant cells in the population. Gouma et al. (2015b) noticed ~0.2 and 0.5 log CFU/mL reduction of *S. cerevisiae* STCC 1172 in apple juice (pH: 3.6) after 3.5 min MH at 50 and 55 °C, respectively. After the same exposure time of 3.5 min in verjuice, a comparatively higher reduction (2.4 log CFU/mL) of acid-adapted *S. cerevisiae* resulted for MH47 (Figure 5.7). Carrillo et al. (2018) achieved 2.6 log CFU/mL reduction of *S. cerevisiae* (KE162) in carrot–orange juice blend (pH: 3.8, 9.8 °Brix) heated at 50 °C for 15 min. In this study, a similar log reduction (2.6 log) of acid-adapted *S. cerevisiae* for

the same treatment time was attained by heating verjuice at between 45 and 47 °C (Figure 5.7). Gabriel (2012) reduced the number of *S. cerevisiae* (BFE-39) in apple juice (pH: 3.7, 12.5 °Brix) by 1 log after exposure to 55 °C for 6.2 min ($D_{55\ °C}$ = 6.2 min). A justification for the relatively higher reduction of *S. cerevisiae* at lower MH temperatures or shorter time in the current study of verjuice could be that the acidic conditions (pH 2.6) and low sugar content (3.7 °Brix) were additive factors in the inactivation and a different strain of *S. cerevisiae* (NRRL Y-139) was used. Shearer, Mazzotta, Chuyate, and Gombas (2002) described the heat resistance of *S. cerevisiae* in various juices as dependent on the composition, as well as pH, as no consistent trend was evidenced solely as a function of pH. Several literature studies also commented that the heat-resistant of yeast cells in fruit juices increased with an increase in the brix value (Beuchat, 1982; Juven, Kanner, & Weisslowicz, 1978; Torreggiani & Toledo, 1986).

It was evident that MH alone produced a moderate inactivation of *S. cerevisiae* and it was insufficient for pasteurization of verjuice based on the FDA requirement (5D reduction). Therefore, it was decided to combine MH and PUV simultaneously, to increase the efficacy of the PUV system for verjuice pasteurization.

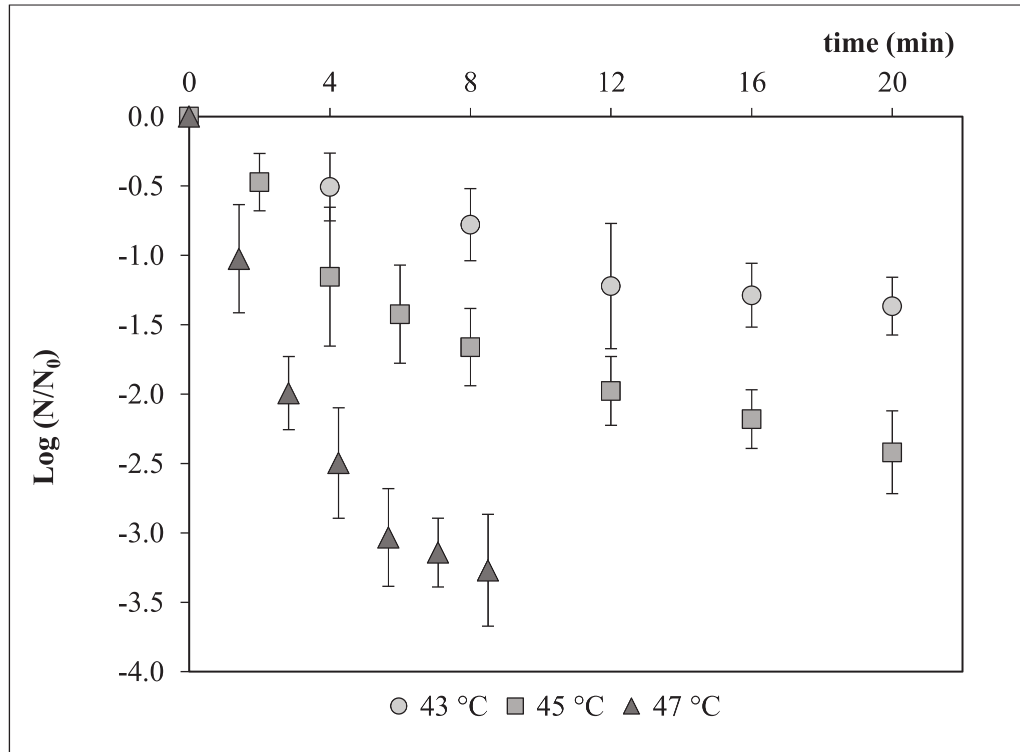


Figure 5.7. The inactivation of acid-adapted *S.cerevisiae* Y139 in verjuice by MH treatment (O: 43 °C [MH43], □: 45 °C [MH45], Δ: 47 °C [MH47])

5.2.4. The Effect of Combined PUV-Mild Heat (PUV+MH) Treatment on *S. cerevisiae* Inoculated into Verjuice

Table 5.5 and Figure 5.8 (a and b) present the log reductions of acid-adapted *S. cerevisiae* (NRRL Y-139) inoculated into verjuice, by PUV+MH using 50 pulses (20 min) at 43 °C, 50 pulses (20 min) at 45 °C and 18 pulses (8.5 min) at 47 °C. These combinations, i.e., PUV+MH43-3, PUV+MH45-3, PUV+MH47-3 for 3 mm juice depth and PUV+MH43-5, PUV+MH45-5, PUV+MH47-5 for 5 mm juice depth, were applied at a constant distance (10 cm) from the lamp.

Table 5.5. The log reductions of acid-adapted *S.cerevisiae* (NRRL Y-139) in verjuice by PUV+MH treatment at different temperatures and juice depth

Treatment code	PUV dose (J/cm^2)	Temperature (°C)	Treatment time (min)	Juice layer depth (mm)	Log reduction ($\log N_0/N$)
PUV+MH43-3	17	43	20	3	2.88 ±0.63c
PUV+MH43-5	17	43	20	5	2.51±0.22c
PUV+MH45-3	17	45	20	3	5.10 ±0.24a
PUV+MH45-5	17	45	20	5	4.25 ±0.46b
PUV+MH47-3	6.17	47	8.5	3	5.06 ±0.08a
PUV+MH47-5	6.17	47	8.5	5	4.36 ±0.25b

Results were presented as "means± standard error". The least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p \leq 0.05$).

In Figure 5.8a, the pasteurization requirement by the FDA (5D reduction) was achieved for 3 mm of verjuice at 45 and 47 °C (PUV+MH45-3 and PUV+MH47-3, respectively), with *S. cerevisiae* reductions of 5.10 ±0.24 log CFU/mL after 50 pulses within 20 min (PUV dose 17 J/cm^2) and 5.06±0.08 log CFU/mL after 18 pulses within 8.5 min (PUV dose 6.12 J/cm^2). These results were not statistically different from each other ($p>0.05$). In comparison, under the same conditions except at a slightly lower temperature at 43 °C (PUV+MH43-3), the yeast inactivation was significantly less, i.e. 2.88±0.63 log CFU/mL at 17 J/cm^2 ($p \leq 0.05$) (Table 5.5).

Figure 5.8b shows that the inactivation of *S. cerevisiae* at 45 and 47 °C was significantly lower in the verjuice having 5 than 3 mm depth ($p \leq 0.05$). The maximal

reduction of yeast cells (4.36±0.25 log CFU/mL) was attained in the verjuice having 5 mm depth after 18 pulses within 8.5 min (6.12 J/cm^2) at 47 °C (PUV+MH47-5). Instead, for the PUV treatment at sub-lethal temperature (43 °C for 20 min at 17 J/cm^2), the juice layer depth was not very important in the inactivation of *S. cerevisiae*, i.e. 2.88 and 2.51 log CFU/mL reductions achieved for 3 (PUV+MH43-3) and 5 mm (PUV+MH43-5) verjuice depths, respectively (Table 5.5).

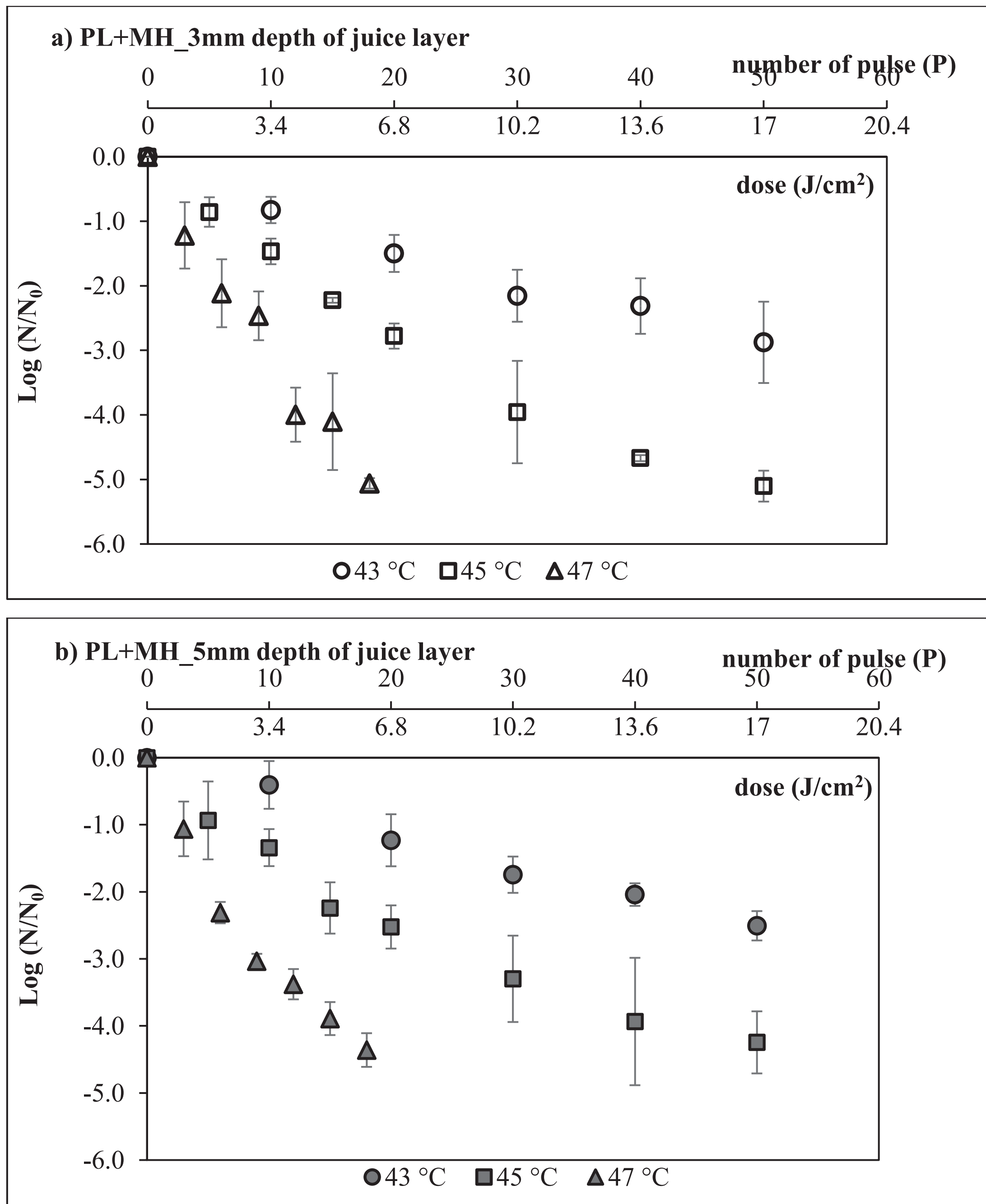


Figure 5.8. The inactivation of acid-adapted *S.cerevisiae* in verjuice by PUV+MH treatment at different juice depth a) 3 mm, b) 5 mm (O: 43 °C, □: 45 °C, Δ: 47 °C)

Therefore, the juice layer depth and MH temperature were influential factors in reducing *S. cerevisiae* (NRRL Y-139) in verjuice when using PUV+MH, with the best efficacy accomplished at low juice depths. A synergistic interaction between PUV and MH has already been depicted by Hilton et al. (2017) on the inactivation of *L. innocua* in buffer solutions, when the PUV was applied at above 40 °C, and by Marquenie et al. (2003) on the inactivation of *Botrytis cinerea* and *Monilinia fructigena* in nutrient agar at 41, 43 and 45 °C. In work by Marquenie et al. (2003), the treatment exposure was decreased compared to the individual treatments. For example, MH inactivation of *B. cinerea* at 45 °C required 10 min, whereas only 3 min was needed when applying 45 °C with 2 min PUV. Although no publications are yet available on the inactivation of yeasts in fruit juices by using combined PUV+MH, the lethal effect of PUV is based on the UV-C region (Koutchma, 2008), and the combined effect of UV-C and MH on the inactivation of *S. cerevisiae* in fruit juices has been established (Gouma et al., 2015b; Carrillo et al., 2018). *S. cerevisiae* was reduced by 5D in apple juice after a UV dose of 2.90 J/mL (2.7 min) at 57.5 °C (Gouma et al., 2015b) and by 4.7D in carrot–orange juice blend after a UV-C dose of 10 kJ/m^2 (15 min) at 50 °C (Carrillo et al., 2018).

It was decided to use a juice depth of 3 mm because verjuice was pasteurized at this level (>5D reduction; Table 5.5). For verjuice pasteurization, the PUV+MH condition was selected as 18 pulses within 8.5 min at 47 °C (PUV+MH47-3) because the visual color of verjuice was minimally changed during the low exposure time of PUV (6.12 J/cm^2) at this temperature.

5.2.5. The Shelf Life of Verjuice Pasteurized by Combined Pulsed-UV Light–Mild Heating (PUV+MH)

5.2.5.1. The Quality Properties of Verjuice Pasteurized by Combined Pulsed-UV Light–Mild Heating at 47 °C (PUV+MH47)

Table 5.6 and 5.7 compare the microbiological, physicochemical (pH, TSS content, TA), optical (absorbance, turbidity, color) and DPPH antioxidant activity properties of the PUV+MH47 pasteurized verjuice (total dose of 6.17 J/cm^2 at 47 °C for

8.5 min) with the controls (P and U). All the data were evaluated by Tukey's comparison test using ANOVA at a 95% confidence interval.

Table 5.6. Physicochemical and optical properties of untreated (U), treated with combined PUV-mild heat (PUV+MH47) and thermally pasteurized (P) verjuice

Treatment code	pH		TSS (°Brix)		TA (%)		Turbidity (NTU)		Abs. coef. (cm^{-1})	
U	2.69	±0.02a	4.20	±0.00b	2.59	±0.03b	158.0	±1.73b	37.78	±0.72b
PUV+MH47	2.67	±0.01a	4.53	±0.06a	2.83	±0.02a	194.0	±6.56a	41.61	±0.83a
P	2.67	±0.01a	4.10	±0.00c	2.59	±0.03b	193.3	±5.51a	36.62	±0.58b

Results were presented as "means± standard error". The least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p \leq 0.05$). TSS: Total Soluble Solid content, TA: Titratable acidity, Abs. coef.: Absorption coefficient.

According to Table 5.6, all verjuice samples had similar pH values to each other ($p > 0.05$). Likewise, Caminiti, Noci, Morgan, Cronin, and Lyng (2012a) found no difference between the pH values of orange–carrot juice blend after PUV treatment (3.3 J/cm^2) and pasteurization (72 °C/ 26 s), respectively. Other investigators also noted that PUV treatment did not alter the pH of fruits or fruit juices (Kwaw et al., 2018a; Koh et al., 2016; Maftei et al., 2014; Pataro et al., 2015), TSS and TA (Caminiti et al., 2011b, 2012a; Munoz et al., 2012; Palgan et al., 2011). The TSS and TA of PUV+MH47 pasteurized verjuice (4.53 °Brix, 2.83% TA) were slightly higher than U (4.10 °Brix, 2.59% TA) and P-treated (4.20 °Brix, 2.59% TA) verjuice controls ($p \leq 0.05$). No published studies have yet detected an increase in the TAA of fruit juices after PUV treatment, but both the TSS and TA increases recorded for the combined PUV+MH47-pasteurized verjuice could be associated with the evaporation during the process (47 °C 8.5 min), given the uncovered jacketed beaker used in the verjuice treatment. Gerard and Roberts (2004) discerned a TSS increase in apple juice after heat treatment.

The turbidity values of treated verjuice (PUV+MH47 and P) were similar whereas the negative control was comparatively less turbid ($p \leq 0.05$). The increased turbidity of fruit juices has been linked to heat treatment (Gerard & Roberts, 2004; Kaya et al., 2015; Rivas et al., 2006). Gerard and Roberts (2004) indicated that the increased cloudiness of juice could arise from the interactions of haze-active proteins and haze-active polyphenols, which increase the number of suspended particles in the juice. A

corresponding rise in the absorbance of verjuice was recorded after the combined PUV+MH47 pasteurization, depending on the juice cloudiness. The absorption coefficient was statistically higher for PUV+MH-treated juice (41.61 cm^{-1}) than the U (37.78 cm^{-1}) and P-treated (36.62 cm^{-1}) verjuice controls that were similar. Muller et al. (2014) detected an increase in the absorption coefficients of both apple and grape juice, respectively, after UV-C (254 nm, 100.47 kJ/L) and UV-B (290-315 nm, 71.51 kJ/L) irradiation, which correlated with the color change of the juices.

Table 5.7 and Figure 5.9 display the color properties (L*, a*, b*, C*, h°, ΔE, BI) of U, PUV+MH47 and P verjuice. According to Table 5.7, lower L*, and higher a* and b* values of PUV+MH47-pasteurized verjuice were recorded (L*: 23.74, a*: 0.28, b*: 5.12) than U (L*: 24.91, a*: 0.07, b*: 4.16) and P-treated (L*: 24.42, a*: 0.08, b*: 3.98) verjuice, possibly because the PUV-C and MH at 47 °C induced browning of the juice. Aguilar et al. (2016) detected the browning of nectarine juice (loss of brightness) treated at mild temperatures after UV-C irradiation at 45 °C. Bhat and Stamminger (2015) reported an increase of a* values could be due to the formation of melanoidin pigments in the juice. Also, Muller et al. (2014) determined the browning of apple and grape juices (decrease in L* and increase in a* and b* values) after UV-C irradiation, and stated that browning reactions in juice could occur as a result of the oxidation of phenolic compounds during the UV-C process.

Table 5.7. Color properties of untreated (U), treated with combined PUV-mild heat (PUV+MH47) and thermally pasteurized (P) verjuice

Treatment code	L*	a*	b*	C*	h°
U	24.91 ±0.36a	0.07 ±0.07b	4.16 ±0.11b	4.16 ±0.11b	88.96 ±1.01a
PUV+MH47	23.74 ±0.07b	0.28 ±0.05a	5.12 ±0.04a	5.12 ±0.04a	86.84 ±0.57a
P	24.42 ±0.23ab	0.08 ±0.04b	3.98 ±0.08b	3.98 ±0.08b	88.79 ±0.49a

Results were presented as "means± standard error". The least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p \leq 0.05$).

When comparing the treatments, the C* values of verjuice (Table 5.7), indicated a slight color saturation after the combined PUV+MH47 treatment (C*: 5.12). However, the positive control was less saturated (C*: 3.98) than the negative control (C*: 4.16). A

similar increase of saturation was detected in UV-C irradiated pineapple juice, by Chia et al. (2012). Yellowness was similar among the samples regarding the h° value (p>0.05).

Considering all these modifications in L*, a*, and b* values, the BI of PUV+MH47-pasteurized verjuice was also slightly greater among the samples (Figure 5.9c). Although the ΔE of PUV+MH47-treated (1.54), and P-treated (0.69) verjuice in Figure 5.9b were significantly different, the ΔE in both was only "slightly noticeable", according to the color classification (slightly noticeable: ΔE 0.5-1.5) by Cserhalmi et al. (2006). Kwaw et al. (2018a) also observed slightly noticeable color change after thermal (ΔE: 0.50) and PUV (ΔE: 0.55) pasteurization of the lactic acid fermented mulberry juice. They reported that these color difference could be resulted from the effect of heating and photochemical degradation reactions for thermal and PUV treatments, respectively.

The DPPH antioxidant capacity of the negative (91.50%) and positive controls (90.11%) were not statistically different from the PUV+MH47 (92.18%)-treated verjuice (Figure 5.9a). Likewise, earlier studies did not find a significant alteration in the antioxidant capacity of orange–carrot juice blend after PUV treatment (Caminiti et al., 2012a) and pomegranate and grape juices after PUV-C and thermal heat treatments (Pala & Toklucu, 2011, 2013a).

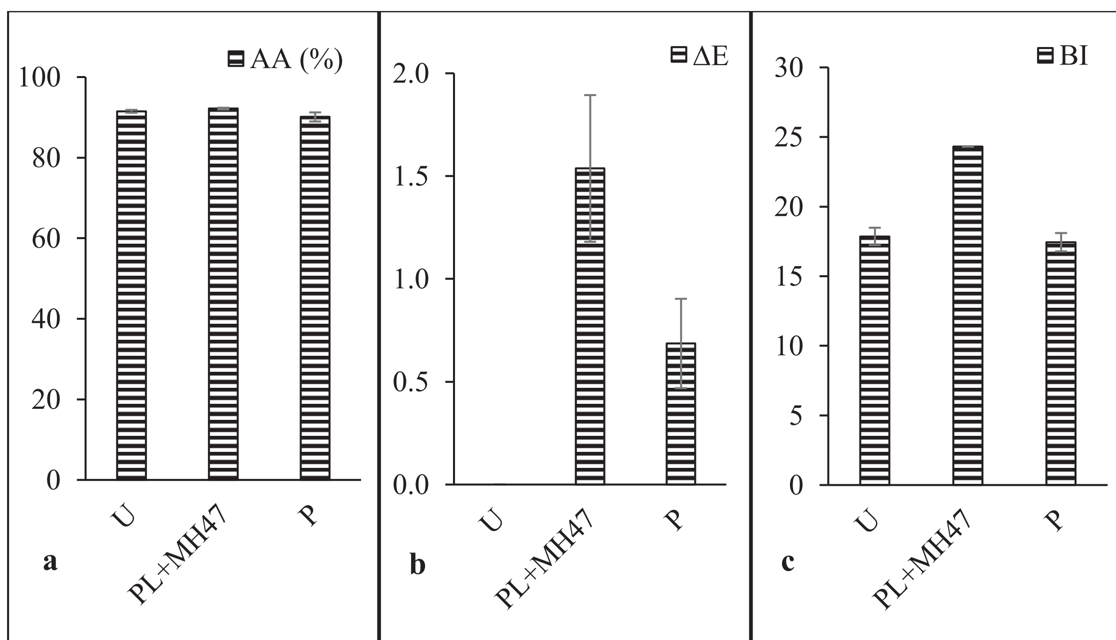


Figure 5.9. Antioxidant activity (AA) (a), Total color difference (ΔE) (b) and browning index (BI) (c) of untreated (U), treated with combined PUV-mild heat (PUV+MH47) and thermally pasteurized (P) verjuice

5.2.5.2. Effect of Combined PUV+MH47 Treatment on the Quality Properties of Verjuice During Storage

Microbiological Properties of Verjuice during Storage

According to the microbial criteria given by the Institute of Food Science and Technology (IFST) and mandated by the Turkish Food Codex (Microbiological Criteria No. 2001/19), the acceptable maximum TAMC and YMC in fruit juice and nectars must be 4 and 3 log CFU/mL, respectively (IFST, 1999; Turkish Food Codex, 2002). Thus, microbiological spoilage of verjuice samples during the shelf life was evaluated based on these microbiological criteria. Logarithmic changes in the TAMC, YMC and TC of the negative control (U), PUV+MH47 (6.17 J/cm^2, 47 °C, 8.5 min)-pasteurized and positive control (P; 72 °C for 18 s) during 6 weeks of refrigerated (4.94±0.89 °C) and room temperature (25 °C) storage were assessed.

According to the microbiological criteria, the negative controls (U) were completely spoiled at room conditions (25 °C) by increasing the YMC from the initial load of 0.77±0.68 to 3.70±0.80 log CFU/mL (>3 log) within 2 weeks. However, no growth was detected in treated (PUV+MH47, P) verjuice during 6 weeks of room storage. Considering the refrigerated (4.94±0.89 °C) verjuice samples, no microbial growth occurred in all samples throughout 6 weeks. Thus, the shelf life of verjuice could be increased by applying a suitable process and storing at refrigerated temperature.

The use of pulsed-light technology to increase the shelf life of fruit juices has been examplified in the literature. Ferrario and Guerrero (2016) applied PUV+US to natural apple juice to control the YMC during 6 days under cold conditions (4 °C), whereas the count was increased by 2 log in untreated juice. In Ferrario et al. (2015), *S. cerevisiae* increased (~1.5 log) in untreated apple juice after 7 days at 5 °C, but no growth was detected in PUV-treated juice (71.6 J/cm^2). Koh et al. (2016) increased the shelf life of cantaloupe fruit up to 28 days (based on the microbial limit [6 log] without quality loss), by applying repetitive PUV (0.9 J/cm^2) to the fruit every 2 days, when compared to the untreated fruit that had a shelf life of 8 days at 4 °C. PUV-C also has a crucial role in the decontamination of food and shelf life extension. Several studies also reported that shelf life of various fruit juices was extended a few days by applying UV-C irradiation under

refrigerated conditions (Kaya et al., 2015; Pala & Toklucu, 2013b; Tran & Farid, 2004; Unluturk & Atilgan, 2015).

Consequently, verjuice pasteurized with combined PUV+MH47 was microbiologically safe after 6 weeks of storage under refrigerated (4.94±0.89 °C) and room temperature (25 °C) conditions. There were no microorganisms in its composition at the end of the 6 weeks. The microbiological safety could be attributed to the lack of initial microbial count in its composition, its highly acidic conditions and the synergistic effect of PUV and MH.

Physicochemical, Optical and Antioxidant Activity Properties of Verjuice during Storage

Table 5.8 and 5.9 demonstrate some physicochemical, optical (pH, total TSS, TA, absorption coefficient, turbidity and color parameters) and DPPH antioxidant activity properties of the controls (U and P [72 °C, 18 s]) and PUV+MH47-pasteurized (6.17 J/cm^2, 47 °C, 8.5 min) verjuice during 6-week shelf life under refrigerated (4.94±0.89 °C) and room temperature (25 °C) conditions. One-way ANOVA analysis and Tukey's comparison test at a 95% confidence interval were applied to the data of all analyses. Changes in the pH, TSS, TA, absorption coefficient, turbidity (Figure 5.10) and DPPH antioxidant activity and color parameters (L*, a*, b*, C*, h°, ΔE, BI) (Figure 5.11) during 6 weeks were also investigated.

Although there was a statistically significant difference between the values given in Table 5.8, it was understood from Figure 5.10 (a, b, c) that some quality properties of all the juices, i.e., pH, TSS, TA and DPPH antioxidant activity, did not dramatically change during the refrigerated and room temperature storage for 6 weeks. For verjuice pasteurized with PUV+MH47, the measurement ranges were pH 2.67-2.76, 4.53-4.40 °Brix and 2.83-3.09% TA during refrigerated storage, and pH 2.67-2.78, 4.53-4.40 °Brix and 2.83-3.12% TA, when stored at room temperature. The TA was constant until week 4 and then began to increase slightly at week 6, for both storage temperatures (Figure 5.10c). Koh et al. (2016) documented no change in pH, TSS and TA of PUV-treated cantaloupe during 28 days storage at 4 °C. Salinas-Roca, Soliva-Fortuny, Welti-Chanes, and Martin-Belloso (2016) found a slight but unimportant change in pH and TSS of PUV-treated fresh-cut mango after 14 days storage at 4 °C. Elsewhere, these physicochemical

values were generally unchanged during the shelf life of fruit juices treated with PUV-C and its combined technologies (La Cava & Sgroppo, 2015; Riganakos et al., 2017; Tandon et al., 2003).

Turbidity did not change until 2 weeks for both storage conditions, then cloudiness of the juice significantly increased from 158 to 254 NTU for U, from 194 to 345 NTU for PUV+MH47 and from 193 to 309 NTU for P verjuice samples until 6 weeks under refrigerated conditions ($p \leq 0.05$) (Figure 5.10d). This modification might be related to the formation of tartrate crystals due to the inefficient detartarisation step (Andres et al., 1997; Cemeroglu, 2004). Conversely, combined PUV+MH47-treated and P-treated verjuice samples stored at 25 °C drastically turned cloudy and were unacceptable at 6 weeks shelf life (Figure 5.10d). The turbidity of the negative control was not measured after week 2 at 25 °C because it had completely spoiled. The cloudiness in verjuice stored at room temperature could be explained by the development of the moulds within the juice during its shelf life. For the treated verjuice samples at 25 °C, proteins and phenolic compounds in the juice composition could form complexes, leading to cloudiness in the juice (Lee et al., 2007). Additionally, the increment of turbidity in fruit juices could be explained by the inadequate inactivation of pectin methyl esterase enzyme, which is responsible for juice cloudiness (Rivas et al., 2006).

There was a fluctuation throughout 6 weeks in the absorption coefficients of all untreated and treated samples (U, PUV+MH47, P) stored at refrigerated and room temperature (Figure 5.10e). The change from the initial (week 0) to the end (week 6) of refrigerated storage was not very noticeable for the negative control (from 37.78 to 38.30 cm^{-1}) and the PUV+MH47-treated verjuice (from 41.61 to 42.99 cm^{-1}) relative to positive control (from 36.62 to 40.47 cm^{-1}). Considering verjuice samples stored at room temperature, the absorption coefficient dramatically increased at week 6 for the combined PUV+MH47 juices (48.73 cm^{-1}) and positive controls (44.47 cm^{-1}). Guerrero-Beltran and Barbosa-Canovas (2004) indicated a relationship between suspended particles and the absorptivity of fruit juices, i.e. higher cloudiness of verjuice could result in a higher absorption coefficient and less light transmittance. The increase of absorbance in fruit juices during its shelf life could result from the decomposition of colored compounds in the juice composition and the formation of dark-colored pigments (melanin and melanoidins) by browning reactions (Muller et al., 2014; Unluturk & Atilgan, 2015). Additionally, the absorption properties of melanin pigment to UV-C light was reported in literature (Seiji & Iwashita, 1965).

Table 5.8. The effect of storage (4 °C) on the physicochemical, optical and antioxidant properties of untreated verjuice & verjuice subjected to different treatments

		4 °C				
week		**0**	**1**	**2**	**4**	**6**
pH	**U**	2.69 ±0.02*a*	2.71 ±0.01*a*	2.74 ±0.01*b*	2.77 ±0.00*c*	2.76 ±0.01*bc*
	PUV+MH47	2.67 ±0.01A	2.70 ±0.01B	2.73 ±0.01C	2.76 ±0.01D	2.76 ±0.01D
	P	2.67 ±0.01a	2.71 ±0.01b	2.73 ±0.01c	2.78 ±0.01d	2.77 ±0.01d
TSS (°Brix)	**U**	4.20 ±0.00*a*	4.20 ±0.00*a*	4.17 ±0.06*a*	4.23 ±0.06*a*	4.17 ±0.06*a*
	PUV+MH47	4.53 ±0.06AB	4.50 ±0.00A	4.47 ±0.06AC	4.60 ±0.00B	4.40 ±0.00C
	P	4.10 ±0.00a	4.20 ±0.00ab	4.10 ±0.00a	4.23 ±0.06b	4.07 ±0.06a
TA (%)	**U**	2.59 ±0.03*a*	2.60 ±0.01*a*	2.55 ±0.02*a*	2.59 ±0.03*a*	2.86 ±0.01*b*
	PUV+MH47	2.83 ±0.02A	2.78 ±0.03AB	2.75 ±0.01B	2.76 ±0.02B	3.09 ±0.01C
	P	2.59 ±0.03a	2.59 ±0.03a	2.51 ±0.01b	2.54 ±0.01ab	2.83 ±0.03c
Turbidity (NTU)	**U**	158.0 ±1.7*a*	176.0 ±9.5*a*	194.0 ±7.0*ab*	220.0 ±10.1*bc*	253.7 ±29.9*c*
	PUV+MH47	194.0 ±6.6A	229.0 ±8.7B	265.0 ±2.6C	309.3 ±7.4D	345.3 ±20.7E
	P	193.3 ±5.5a	216.0 ±6.0b	238.3 ±4.5c	274.3 ±9.5d	309.3 ±4.2e
Abs. coef. (cm^{-1})	**U**	37.78 ±0.72*a*	38.14 ±0.71*a*	38.11 ±0.80*a*	38.98 ±1.05*a*	38.30 ±1.47*a*
	PUV+MH47	41.61 ±0.83A	45.77 ±0.38B	38.11 ±0.80C	43.91 ±0.90BD	42.99 ±0.76AD
	P	36.62 ±0.58a	38.25 ±1.18ab	37.20 ±0.40ab	38.81 ±1.14ab	40.47 ±2.19b
Color						
L*	**U**	24.91 ±0.36*a*	25.60 ±0.20*ab*	25.56 ±0.10*ab*	25.98 ±0.25*b*	24.03 ±0.60*a*
	PUV+MH47	23.74 ±0.07A	24.96 ±0.22B	24.23 ±0.21A	25.05 ±0.28B	23.69 ±0.24A
	P	24.42 ±0.23a	25.43 ±0.22b	25.29 ±0.31b	25.49 ±0.23b	23.84 ±0.40a
a*	**U**	0.07 ±0.07*a*	0.20 ±0.01*a*	0.33 ±0.03*b*	0.48 ±0.05*c*	0.67 ±0.05*d*
	PUV+MH47	0.28 ±0.05A	0.43 ±0.03B	0.60 ±0.03C	0.62 ±0.01C	0.80 ±0.08D
	P	0.08 ±0.04a	0.22 ±0.01b	0.32 ±0.02c	0.44 ±0.01d	0.64 ±0.02e
b*	**U**	4.16 ±0.11*a*	4.06 ±0.04*a*	4.09 ±0.04*a*	4.12 ±0.06*a*	4.71 ±0.22*b*
	PUV+MH47	5.12 ±0.04A	4.36 ±0.11B	4.86 ±0.08A	4.42 ±0.12B	4.54 ±0.12B
	P	3.98 ±0.08ab	3.57 ±0.08a	3.88 ±0.13a	3.93 ±0.04ab	4.37 ±0.31b
C*	**U**	4.16 ±0.11*a*	4.06 ±0.04*a*	4.10 ±0.04*a*	4.14 ±0.07*a*	4.76 ±0.22*b*
	PUV+MH47	5.12 ±0.04A	4.38 ±0.11B	4.90 ±0.08A	4.46 ±0.12B	4.61 ±0.10B
	P	3.98 ±0.08a	3.58 ±0.08a	3.89 ±0.13a	3.95 ±0.04a	4.41 ±0.30b
h°	**U**	88.96 ±1.01*a*	87.16 ±0.07*b*	85.33 ±0.45*c*	83.35 ±0.73*d*	81.94 ±0.24*d*
	PUV+MH47	86.84 ±0.57A	84.34 ±0.21B	82.91 ±0.31BC	82.03 ±0.16C	79.98 ±1.26D
	P	88.79 ±0.49	86.51 ±0.21	85.21 ±0.34	83.59 ±0.22	81.67 ±0.48
ΔE	**U**	0.00 ±0.00*a*	0.71 ±0.53*ab*	0.71 ±0.46*ab*	1.14 ±0.44*b*	1.24 ±0.45*b*
	PUV+MH47	0.00 ±0.00A	1.44 ±0.20B	0.65 ±0.14C	1.52 ±0.33B	0.79 ±0.14C
	P	0.00 ±0.00a	1.10 ±0.26b	0.92 ±0.32b	1.13 ±0.06b	0.94 ±0.31b
BI	**U**	17.86 ±0.62*a*	17.25 ±0.33*a*	17.78 ±0.18*a*	18.02 ±0.26*a*	23.16 ±1.32*b*
	PUV+MH47	24.32 ±0.02A	19.84 ±0.78B	23.48 ±0.64A	20.59 ±0.82B	23.08 ±0.32A
	P	17.44 ±0.66a	15.23 ±0.48a	17.02 ±0.81a	17.43 ±0.28a	21.56 ±1.95b
AA (%)	**U**	91.50 ±0.35*ab*	92.34 ±1.40*ab*	93.58 ±0.00*a*	91.88 ±0.78*ab*	91.06 ±1.07*b*
	PUV+MH47	92.18 ±0.21AB	93.44 ±0.21A	93.28 ±0.00AB	91.48 ±0.86B	91.17 ±1.41B
	P	90.11 ±1.13a	93.31 ±0.68b	93.38 ±0.09b	91.62 ±0.26ab	92.50 ±0.45b

Results were presented as "means± standard error". The least significant difference was determined by Tukey pairwise comparison test. Different italic (U), capital (PUV+MH47) and small case (P) letters in the same column show the significant differences in the properties between means of weeks during shelf life study ($p \leq 0.05$).

Table 5.9. The effect of storage (25 °C) on the physicochemical, optical and antioxidant properties of untreated verjuice & verjuice subjected to different treatments

		25 °C									
week		**0**		**1**		**2**		**4**		**6**	
pH	**U**	2.69	±0.02*a*	2.68	±0.00*a*	2.72	±0.03*a*			Spoiled	
	PUV+MH47	2.67	±0.01A	2.68	±0.01A	2.74	±0.01B	2.77	±0.00C	2.78	±0.01C
	P	2.67	±0.01a	2.69	±0.00a	2.75	±0.01b	2.78	±0.01c	2.78	±0.01c
TSS (°Brix)	**U**	4.20	±0.00*a*	4.33	±0.23*a*	4.03	±0.40*a*			Spoiled	
	PUV+MH47	4.53	±0.06AB	4.37	±0.06A	4.50	±0.10AB	4.63	±0.06B	4.40	±0.00A
	P	4.10	±0.00a	4.17	±0.06a	4.13	±0.06a	4.23	±0.06a	4.10	±0.10a
TA (%)	**U**	2.59	±0.03*a*	3.18	±1.03*a*	2.51	±0.14*a*			Spoiled	
	PUV+MH47	2.83	±0.02A	2.82	±0.04A	2.79	±0.01A	2.79	±0.03A	3.12	±0.01B
	P	2.59	±0.03a	2.62	±0.03a	2.57	±0.01a	2.56	±0.02a	3.07	±0.28b
Turbidity (NTU)	**U**	158.0	±1.7*a*	160.0	±10.6*a*	199.3	±32.7*a*			Spoiled	
	PUV+MH47	194.0	±6.6A	200.3	±4.0A	231.3	±4.0A	422.2	±34.8B	1018	±31.1C
	P	193.3	±5.5a	205.0	±2.0a	224.3	±1.5a	360.3	±4.7b	805.7	±45.1c
Abs. coef. (cm^{-1})	**U**	37.78	±0.72*a*	40.66	±1.69*a*	38.61	±3.16*a*			Spoiled	
	PUV+MH47	41.61	±0.83A	46.36	±1.00B	44.53	±1.13AB	47.63	±1.58B	48.73	±0.57B
	P	36.62	±0.58a	39.53	±1.87b	39.26	±0.18b	43.55	±0.66c	44.47	±0.70c
Color											
L*	**U**	24.91	±0.36*a*	24.71	±0.25*a*	23.49	±1.09*a*			Spoiled	
	PUV+MH47	23.74	±0.07A	24.49	±0.35B	23.85	±0.08A	23.61	±0.06A	25.57	±0.35C
	P	24.42	±0.23a	24.57	±0.19a	24.28	±0.07a	23.47	±0.05b	22.17	±0.08c
a*	**U**	0.07	±0.07*a*	0.80	±0.05*b*	1.01	±0.36*b*			Spoiled	
	PUV+MH47	0.28	±0.05A	1.23	±0.02B	1.59	±0.03C	1.17	±0.04B	1.44	±0.03D
	P	0.08	±0.04a	0.75	±0.05b	1.08	±0.05c	1.27	±0.01d	1.40	±0.03e
b*	**U**	4.16	±0.11*a*	5.66	±0.35*a*	5.63	±1.12*a*			Spoiled	
	PUV+MH	5.12	±0.04A	6.05	±0.10B	6.00	±0.03B	4.39	±0.05C	7.15	±0.20D
	P	3.98	±0.08a	5.36	±0.05b	5.88	±0.18c	4.54	±0.05d	5.12	±0.20c
C*	**U**	4.16	±0.11*a*	5.72	±0.36*a*	5.72	±1.16*a*	Spoiled			
	PUV+MH47	5.12	±0.04A	6.18	±0.10B	6.21	±0.04B	4.55	±0.04C	7.29	±0.20D
	P	3.98	±0.08a	5.41	±0.06b	5.98	±0.17c	4.72	±0.05d	5.31	±0.20b
h°	**U**	88.96	±1.01*a*	81.94	±0.35*b*	80.08	±1.83*b*			Spoiled	
	PUV+MH47	86.84	±0.57A	78.52	±0.10B	75.21	±0.20C	75.13	±0.55C	78.63	±0.41B
	P	88.79	±0.49a	81.99	±0.45b	79.62	±0.68c	74.43	±0.19d	74.72	±0.36b
ΔE	**U**	0.00	±0.00*a*	1.69	±0.28*b*	2.66	±0.37*c*			Spoiled	
	PUV+MH47	0.00	±0.00A	1.55	±0.07B	1.59	±0.05B	1.15	±0.11B	2.97	±0.33C
	P	0.00	±0.00a	1.58	±0.14b	2.15	±0.20b	1.63	±0.22b	2.86	±0.22c
BI	**U**	17.86	±0.62*a*	27.59	±2.21*b*	29.55	±5.73*b*			Spoiled	
	PUV+MH47	24.32	±0.02A	31.20	±1.07B	32.98	±0.34C	23.54	±0.21A	35.86	±0.63D
	P	17.44	±0.66a	26.07	±0.61b	30.09	±0.91c	24.77	±0.34a	30.08	±1.14c
AA (%)	**U**	91.50	±0.35*a*	93.44	±0.21*b*	92.49	±1.02*ab*			Spoiled	
	PUV+MH47	92.18	±0.21AB	93.26	±0.52A	92.99	±0.39A	91.24	±0.68B	91.28	±1.02B
	P	90.11	±1.13a	93.26	±0.29b	93.08	±0.23b	91.22	±0.36a	91.45	±0.44a

Results were presented as "means± standard error". The least significant difference was determined by Tukey pairwise comparison test. Different italic (U), capital (PUV+MH47) and small case (P) letters in the same column show the significant differences in the properties between means of weeks during shelf life study ($p \leq 0.05$).

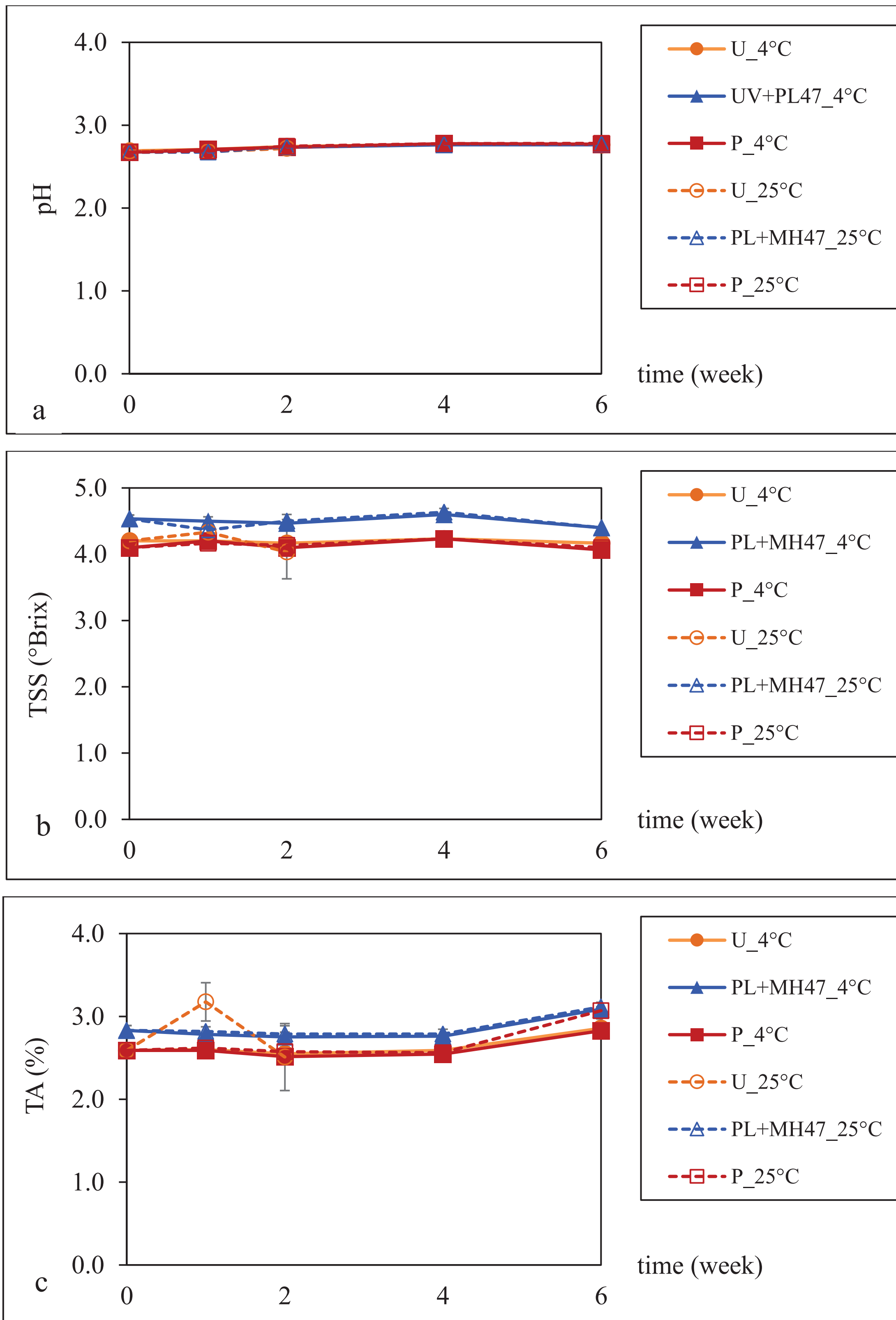
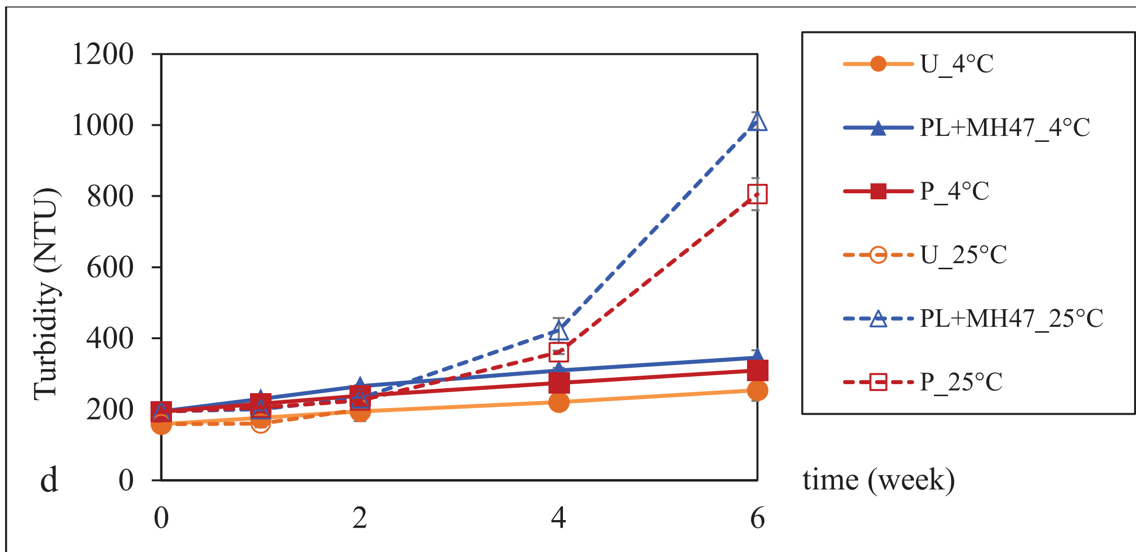
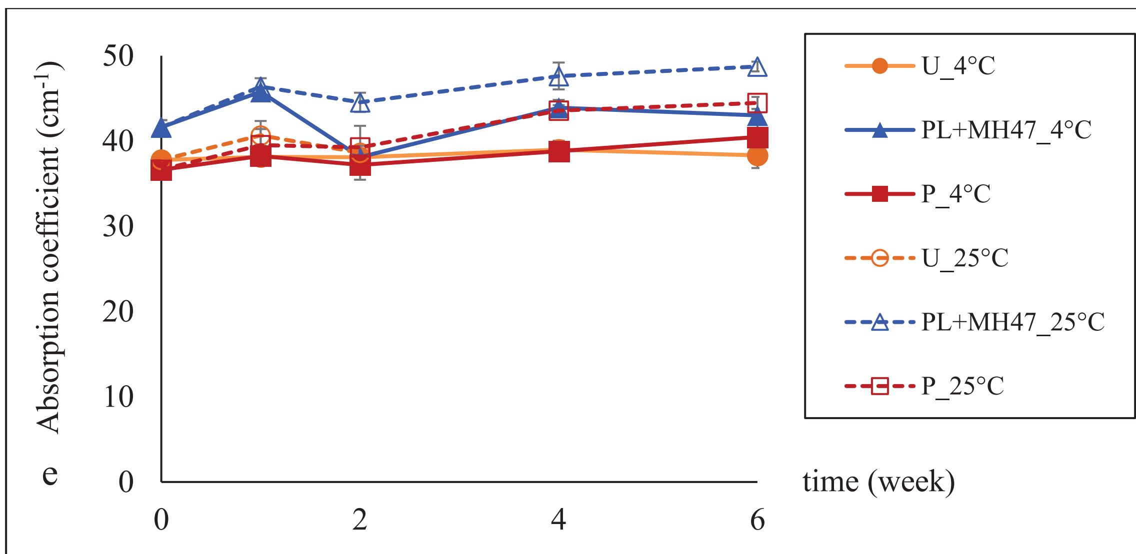


Figure 5.10. Change in physicochemical properties of untreated (U: circle), treated with combination of processes (PUV+MH47: triangle) and thermally pasteurized (P: square) verjuice during 6 weeks at storage of 4°C and 25 °C (a:pH, b:Total soluble solid content, c: Titratable acidity, d: Absorption coefficient, e: Turbidity, f: Antioxidant activity) (4 °C: solid line, 25 °C: dotted line)

(cont. on next page)





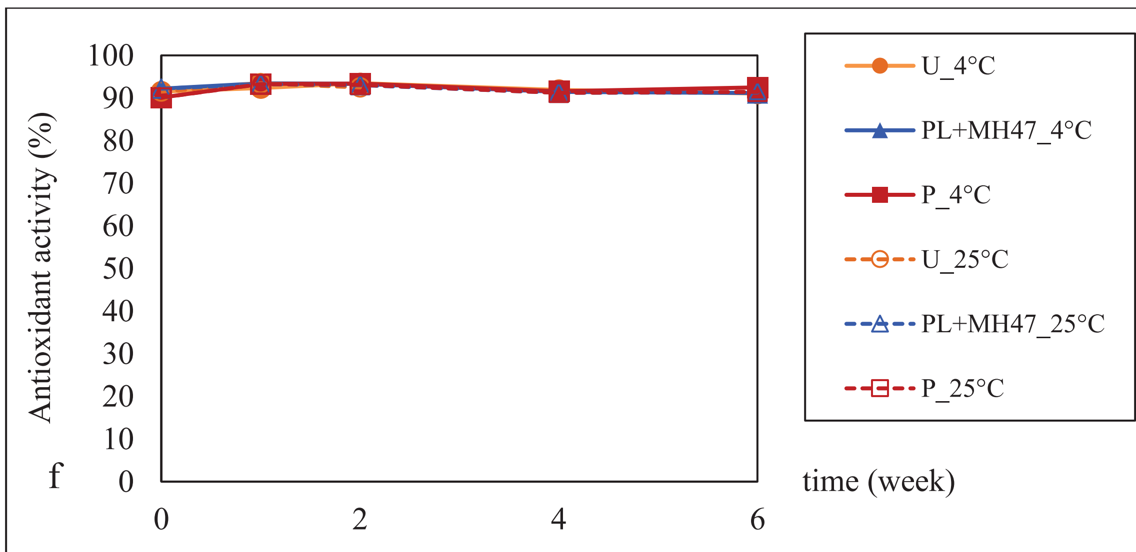


Figure 5.10 (cont.)

The DPPH antioxidant activity of verjuice was very high for all samples, including the controls, in this study (Figure 5.10f). The free radical scavenging activity of U, PUV+MH47 and P verjuice were 91.50, 92.18 and 90.11% at the beginning of the shelf life and did not markedly decrease during storage during storage at refrigeration (4.94±0.89 °C) and room temperature (25 °C). The antioxidant activity of the negative control was 91.06% after refrigeration for 6 weeks while PUV+MH47 verjuice and the positive control exhibited 91.17 and 92.50% at refrigerated, and 91.28 and 91.45% at room temperature, for 6 weeks, respectively. Oms-Oliu, Odriozola-Serrano, and Martin-Belloso (2012) noted that UV-C light could cause the increment or decline in the antioxidant activity of fresh plant products, depending on the dose, time and type of fruit. Kwaw et al. (2018b) monitored the antioxidant activity of lactic acid fermented mulberry juice processed by PUV assisted with ultrosonic treatment during 5 and 25 °C storage. They observed a slight decrease of antioxidant activity of the juice during storage, which was directly related to the degradation of phenolic contents. Additionally, the antioxidant properties of the mulberry juice was better preserved in refrigerated storage. Koh et al. (2016) detected no change in the phenolic content of PUV-treated cantaloupe during its shelf life, which could mean the antioxidant activity was unchanged. The unchanged antioxidant activity may be associated with the stability of phenolic contents due to high acidic conditions of verjuice (Kwaw et al., 2018b). Some literature reports correlated an increased antioxidant activity of PUV- or UV-C-treated fruit products with the phenolic content of the product during its shelf life (Gonzalez-Aguilar, Villegas-Ochoa, Martinez-Tellez, Gardea, & Ayala-Zavala, 2007; Oms-Oliu, Aguilo-Aguayo, Martin-Belloso, & Soliva-Fortuny, 2010a; Pataro et al., 2015).

Table 5.8 and 5.9 and Figure 5.11 detail the alterations in the color properties (L*, a*, b*, C*, h°, ΔE, BI) of all verjuice samples, including the controls, during 6 weeks at refrigerated and room temperature conditions. The L* value of all verjuice samples were almost the same until the end of the 6 weeks of refrigerated storage, whereas it changed slightly after 2 weeks at 25 °C. Although this change was statistically significant, it was unnoticeable (Figure 5.11a). The most important variation occurred in a* values (Figure 5.11b). All samples became significantly redder (increased a* values), particularly when stored at room temperature. The PUV+MH-treated verjuice better maintained the green pigmentation in comparison to the controls under both storage conditions, i.e., a* increased 2.84- and 5.1-fold at the end of the 6 weeks for refrigerated and room temperature storage, respectively, while it increased 9-fold for U and 7.5-fold for P

verjuice samples even during refrigerated stored. Based on the b* values (Figure 5.11c), yellowness slightly varied in all verjuice samples at the end of 6 weeks of refrigerated temperature, i.e. yellowness increased by 13% for U, decreased by 11% for PUV+MH47 and increased by 11% for P verjuice samples. These variations in the yellowness of verjuice were not remarkable, whereas at room temperature, yellowness was markedly increased even at 1 week relative to cooled storage for 6 weeks, and there were fluctuations between these times. The h° of verjuice, which was around the yellow region (90°) according to Pathare et al. (2013), decreased by 8-9% for all verjuice samples, including the controls, during 6 weeks at both storage temperatures (Figure 5.11e). However, it was significantly decreased by 16% for the positive control at the end of the 6 weeks at room temperature. It implied that no prominent change in yellowness of the PUV+MH47-treated verjuice had eventuated during 6 weeks at refrigerated or room temperature, based on b* value and h°. The C* change in verjuice was much more noticeable during the storage at room temperature than refrigeration (Figure 5.11d). At the end of 6 weeks, the PUV+MH47-pasteurized verjuice was 10% less saturated after refrigerated storage but 40% more at the room temperature storage, compared to the initial value.

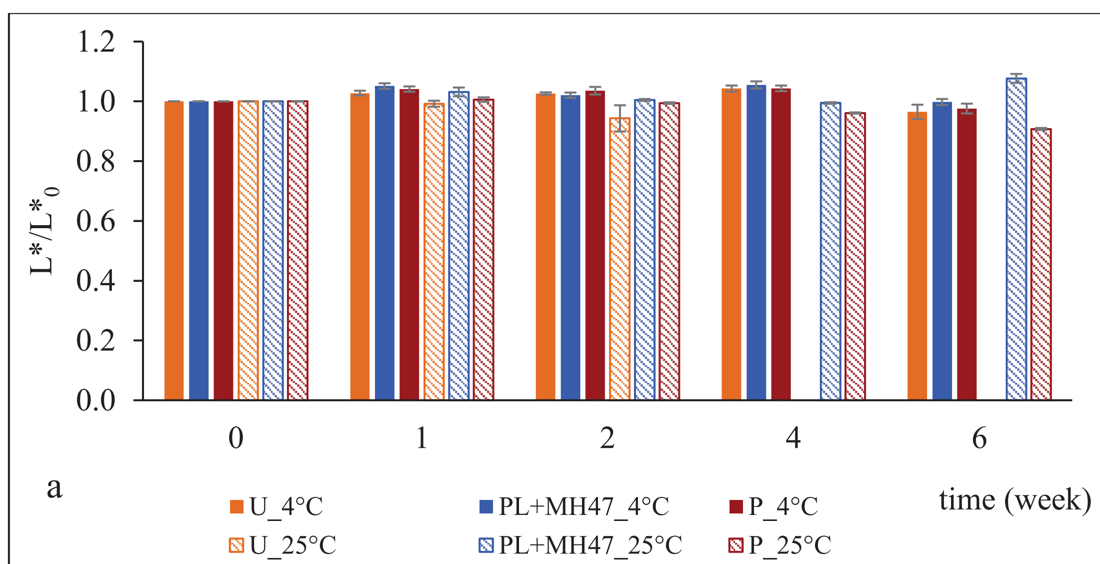
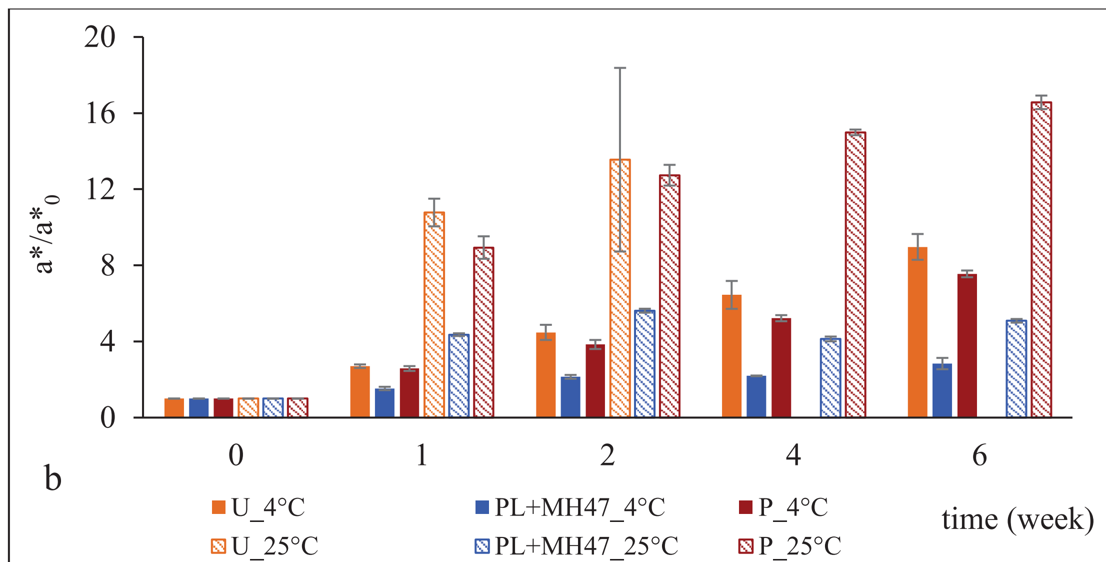
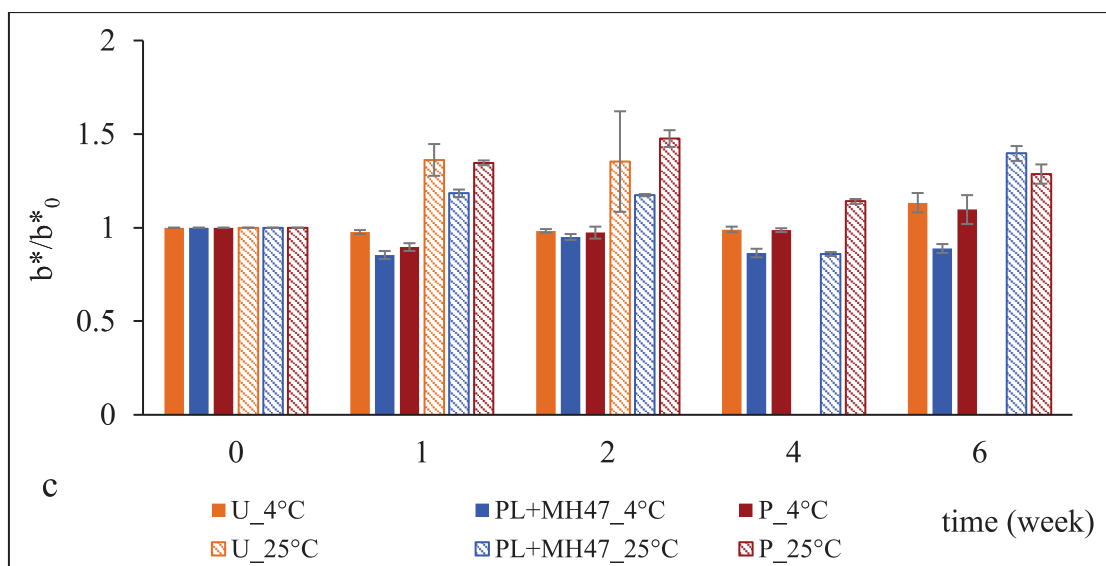


Figure 5.11. Changes in color of untreated (U: circle), combined treated (PUV+MH47: triangle) and thermally (P: square) pasteurized verjuice during 6 weeks at storage of 4 °C and 25 °C (a: lightness-darkness,L*; b: redness-greenness, a*; c: yellowness-blueness, b*; d: hue angle, h; e: chroma, C*; f: total color difference, ΔE; and g: browning index, BI) (4 °C: filled bar; 25 °C: striped bar)

(cont. on next page)





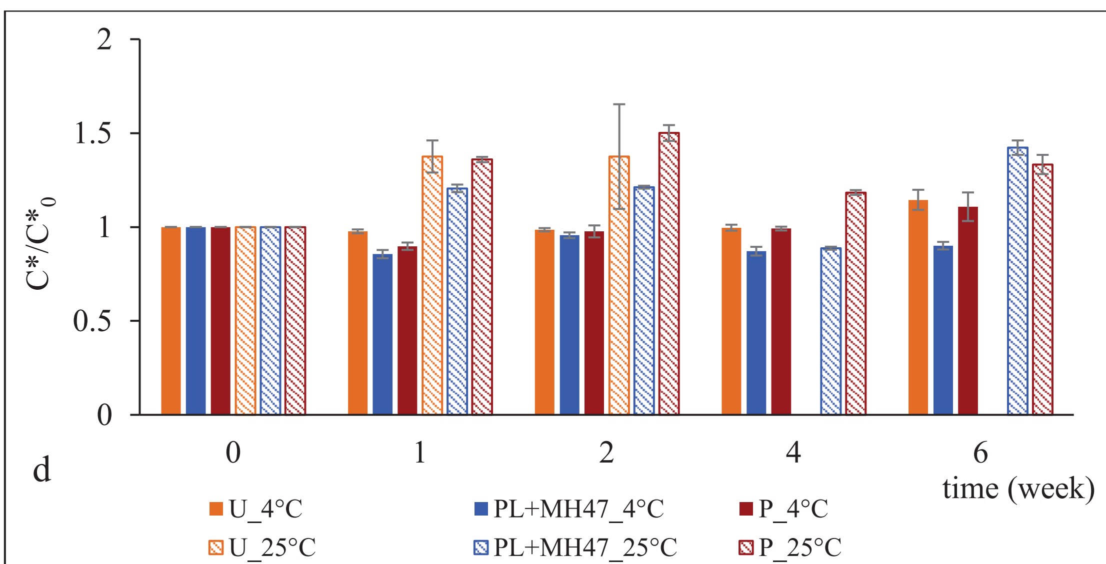


Figure 5.11 (cont.)

(cont. on next page)

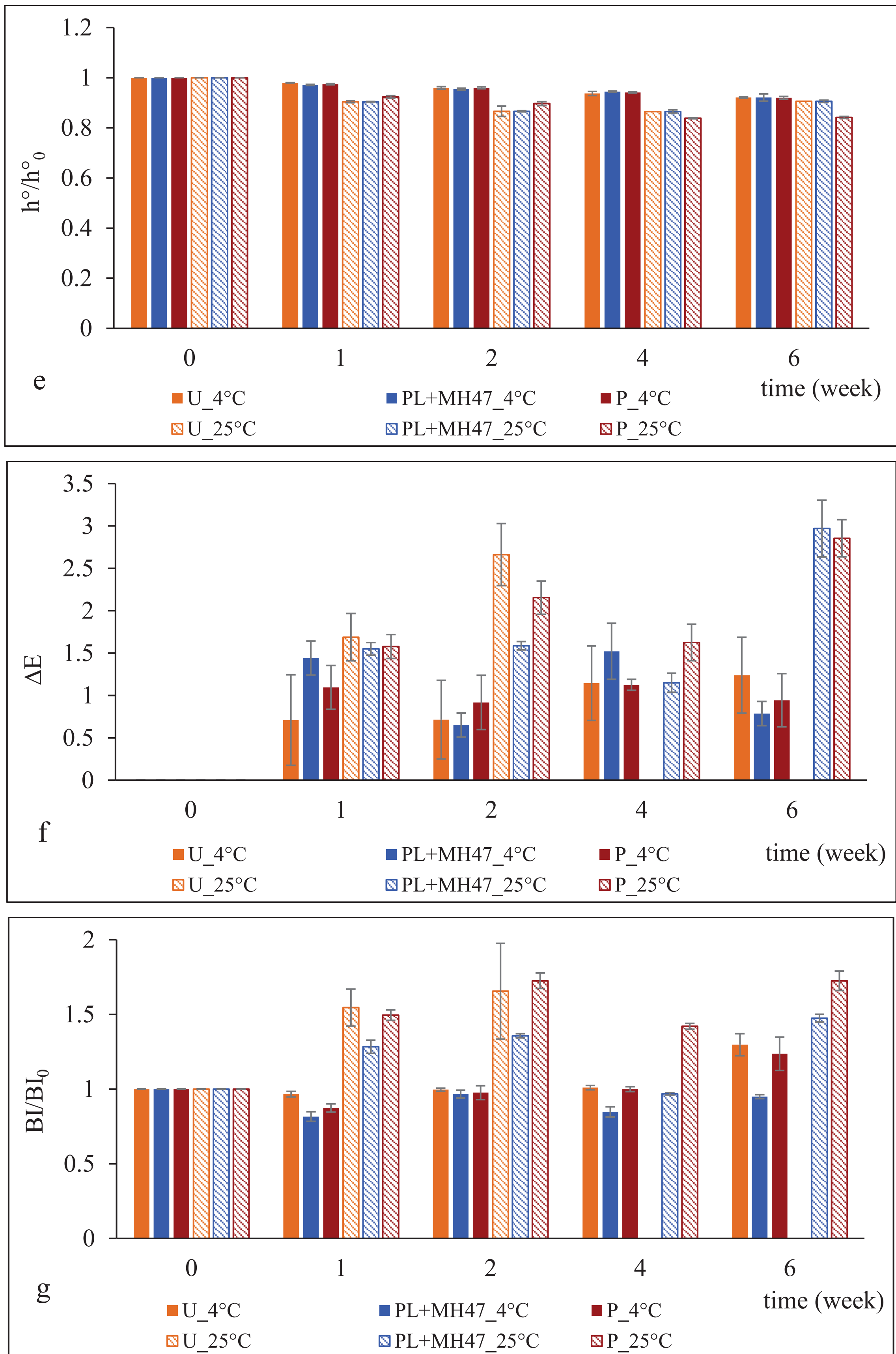


Figure 5.11 (cont.)

The ΔE values of verjuice samples were 1.24 (U), 0.79 (PUV+MH47) and 0.94 (P), which were evaluated as slightly noticeable (0.5-1.5) based on Cserhalmi et al. (2006), at the end of the 6 weeks refrigerated storage (Figure 5.11f). Instead, ΔE increased up to 2.97 (PUV+MH47) and 2.86 (P) after storage for the same time at room temperature, and it was classified as noticeable (1.5-3.0). The ΔE of verjuice during storage could be correlated to the browning reactions. The BI values showed that all samples were maintained better when stored under cooled than room temperature conditions (Figure 5.11g). Besides, the BI for the refrigerated samples at 6 weeks calculated was much higher (24%) in the P verjuice than in the PUV+MH47-pasteurized verjuice.

Generally, L*, b*, h° and C* values of PUV+MH47-treated verjuice was almost maintained at refrigerated conditions, whereas its greenness was degraded (increased a* value). By increasing a* value, its ΔE and BI were also affected. The increased a* value in the verjuice or loss of its greenness could be due to the chlorophyll degradation or generation of brown melanoidin pigments during storage (Bhat & Stamminger, 2015). Ferrario and Guerrero (2016) reported no change in the L* and b* values of natural apple juice after PUV treatment (0.73 J/cm^2) during 12 days of shelf life at cold storage. Koh et al. (2016) did not observe any change in L*, C* and h° of repetitive PUV-treated fresh-cut cantaloupe fruit during 28 days at 4 °C. Salinas-Roca et al. (2016) also did not find a remarkable change in the L* and h° of PUV-treated mango slices after storage for 14 days at 4 °C. The browning or undesirable color of fruit products could occur by interaction of phenolic compounds with oxidative enzymes, such as polyphenol oxidase. PUV treatment could cause a decompartmentalisation in the fruit structure, and then dark-colored melanoidin pigments could form by the interaction between phenolic compounds and the enzyme (Ferrario & Guerrero, 2016). Additionally, several studies mentioned that the PUV could induce non-enzymatic or enzymatic browning in fruit juices (Chia et al., 2012; Ibarz et al., 2005; Kaya et al., 2015). In this study, limited browning occurred in the PUV+MH47-treated verjuice samples stored at refrigerated conditions, due to the restricted the enzyme activity at cold storage.

5.3. Conclusions

In the first part of this chapter, the effects of PUV technology on inactivation of *S. cerevisiae* (NRRL Y-139) cells in verjuice were studied by using different PUV process

parameters in a bench-top PUV equipment, i.e., depth of juice layer (1, 3 and 5 mm), distance from the lamp (5 and 10 cm) and number of pulses (from 0 to 50 pulses). Yeast cells were initially adapted to the acidic conditions of verjuice. The most effective inactivation provided 0.96±0.27 log CFU/mL reductions of *S. cerevisiae* in verjuice, which was achieved by applying PUV to 1 mm juice depth, placed 5 cm from the xenon lamp, using 50 pulses. The calculated PUV dose in this treatment (PUV5-1) was 34 J/cm^2. Thus, PUV treatment alone was insufficient for the pasteurization of verjuice that requires a 5D reduction of the target microorganism, so this technology was combined with MH to increase its inactivation efficiency.

In the second part of this chapter, PUV was combined with three different non-lethal temperatures (43, 45 and 47 °C) to reach a 5-log reduction of *S. cerevisiae* (NRRL Y-139) in verjuice. MH alone was also applied at the same temperatures to evaluate the heating effect on the *S. cerevisiae* cells inoculated into verjuice. Verjuice was incompletely pasteurized by the MH treatments, i.e., the maximum reduction of the acid-adapted *S. cerevisiae* cells was 3.4±0.44 log CFU/mL after exposure to 47 °C for 8.5 min (MH47). However, verjuice was pasteurized for 3 mm depth of verjuice, by applying PUV with 50 pulses (17 J/cm^2, 20 min) at 45 °C and 18 pulses (6.12 J/cm^2, 8.5 min) at 47 °C, respectively. *S. cerevisiae* reductions of 5.10±0.24 and 5.06±0.08 log CFU/mL were obtained by using PUV+MH45-3 and PUV+MH47-3 treatments, respectively. In comparison, the inactivation efficacy of *S. cerevisiae* at 45 and 47 °C was significantly lower in the verjuice having 5 mm depth, which attained a maximal reduction of 4.36±0.25 log CFU/mL after a PUV dose of 6.12 J/cm^2 at 47 °C (PUV+MH47-5). Therefore, the juice layer depth and MH temperature were influential factors in reducing *S. cerevisiae* (NRRL Y-139) in verjuice when using PUV+MH treatment.

In the third part of this chapter, microbiological, physicochemical (pH, TSS, TA), optical (absorbance, turbidity, color) and DPPH antioxidant activity properties of PUV+MH-pasteurized verjuice (3 mm depth of juice layer, 6.12 J/cm^2, 18 pulses, 47 °C for 8.5 min; PUV+MH47-3) were monitored during 6 weeks of refrigerated and room temperature storage conditions, respectively, alongside the positive (heat only at 72 °C/18 s) and negative (no treatment) controls. It was recommended that verjuice should be stored at refrigerated conditions instead of room temperature to better maintain all the juice quality properties. The negative controls were completely spoiled by yeasts and moulds (3.70±0.80 log CFU/mL) within 2 weeks under room conditions (25 °C). No microbial growth occurred in the PUV+MH47-treated verjuice at the end of the 6 weeks

under both storage conditions. Although the physicochemical properties of the PUV+MH47-pasteurized verjuice did not markedly change, the optical properties were affected during the shelf life.

In conclusion, the combined technologies of PUV and MH (hurdle technology) can meet the FDA requirements for pasteurization of verjuice (5-log reduction of target microorganism) and extend the shelf life, by maintaining quality losses to a minimum level. In the future, pulsed-light technology can be developed and used as an effective method for fruit juice pasteurization, by selecting appropriate equipment, optimal process parameters and a suitable juice type.

CHAPTER 6

PRODUCTION OF VERJUICE POWDER

Verjuice is produced from unripe grapes and used as a savoury alternative of vinegar and lemon juice in foods (Oncul & Karabiyikli, 2015). The verjuice is commonly consumed in the form of juice or concentrate and prepared seasonally in a household condition. It has a short shelf life because it is prone to microbiological spoilage. However, the powder form of verjuice can be produced as an alternative souring product with a long shelf life to give aroma in meals, salads and snacks.

Fruit juices are dried in order to reduce weight, volume and the size of their packages and to provide easier transportation, storage stability as well as economic benefits (Goula & Adamopoulos, 2010). The aim of drying is not only to extend the shelf life but also to preserve the quality of fruit juice (Mani et al., 2002). Seasonal fruits and vegetables are usually dried in order to provide the availability of foods all year round (Marques et al., 2006; Ratti, 2001). The excellent nutritional and sensorial quality of the product is provided by freeze drying compared to conventional air drying and spray drying due to low temperature operation (Erbay & Kucukoner, 2008; Ratti, 2001). The freeze drying removes the water from the juice in the ice form by sublimation (Franks, 1998). There were several reported studies related to freeze drying of fruit juices. Chopda and Barrett (2001) produced freeze-dried guava juice powder with a better quality compared to spray-dried powders. Ceballos et al. (2012) analysed some quality parameters of freeze-dried soursop fruit pulp with the addition of maltodextrin. Marques et al. (2006) reported that several types of tropical fruit pulps could be freeze dried with a high nutritional value. Franceschinis et al. (2014) found that the freeze drying method was better than the spray drying to preserve bioactive compounds in blackberry powders. Caparino et al. (2012) studied the refractance window drying, drum drying, spray drying and freeze drying techniques to obtain mango powder, and freeze drying method was identified as the best method preserving quality of powder.

The objective of this chapter was to produce high quality verjuice powder by using freeze drying method. The chapter contains two main steps; as a first, freshly squeezed verjuice was prepared and freeze-dried after the addition of Maltodextrin (DE20) to avoid

stickiness of the powder and increase product yield during drying. Different concentration of maltodextrin (10%, 15% and 20% MD [w/w]) and drying times (FD 36h, 48h and 72h) were performed to produce the optimum quality powder. Product yield and physical, color, thermal, microstructural quality parameters of freeze-dried powders were measured and compared. In the second step, the quality of verjuice powders obtained under the optimum freeze drying conditions were evaluated during the accelerated storage conditions (40 °C/90% RH). For this purpose, some physical properties and color parameters of powders were monitored and compared during the storage period.

6.1. Materials and Methods

6.1.1. Freeze Drying of Verjuice

Freshly squeezed verjuice were obtained from the unripe grapes according to the same preparation procedures described as in the section 3.1.2 of Chapter 3.

The verjuice powder was produced by means of a freeze drying technique and the main steps of production are given in Figure 6.1. Freeze drying process was performed using a laboratory scale freeze dryer (Labconco Equipment Co., Freezone Freeze Dry Systems, Kansas City, MO) located in the Biotechnology and Bioengineering Research and Application Centre in Izmir Institute of Technology. Before drying process, three different concentrations of maltodextrin (MD) having dextrose equivalent of 20 (DE 20) i.e., 10% MD, 15% MD and 20% MD (w/w) were added to the verjuice (4.29±0.07 °Brix) as a drying agent in order to prevent stickiness. Feed mixtures were homogenized for 30 min at room temperature (25 °C). Total soluble solid contents of feed mixtures were measured as 12.97±0.06, 16.63±0.06 and 20.00±0.10 °Brix after addition of 10%, 15% and 20% MD, respectively.

In preliminary studies, verjuice feed mixtures were frozen (24 h/-20 °C) and subjected to freeze drying for 24 h and the fruit juice containing 10%, 15% and 20% MD (feed mixtures) was concentrated up to 36.5, 42.6 and 51 °Brix, respectively. However, 24 h of freeze drying time was not long enough to obtain a powder product. Thus, the freezing time was extended up to 36, 48 and 72 h. After freeze drying process at these processing times, verjuice samples with the same amount of maltodextrin were successfully dried. Feed mixtures were kept in the freezer (-20 °C) for 24 h before being

transferred to the freeze dryer. Condenser temperature and vacuum pressure of the dryer were automatically fixed to the value of -54 °C and 0.014 mbar, respectively. The experimental design for freeze drying of verjuice samples were presented in Table 6.1. After drying process, freeze-dried cakes were milled at 3000 rpm and 10 s by using a grinder (Retsch Grindomix GM200, GmbH & Co, Haan, Germany) to obtain powders. The verjuice powders were sealed in 50 mL airtight falcon centrifuge tubes and transferred into the desiccator at room temperature (25 °C) until use. All drying experiments were repeated three times.

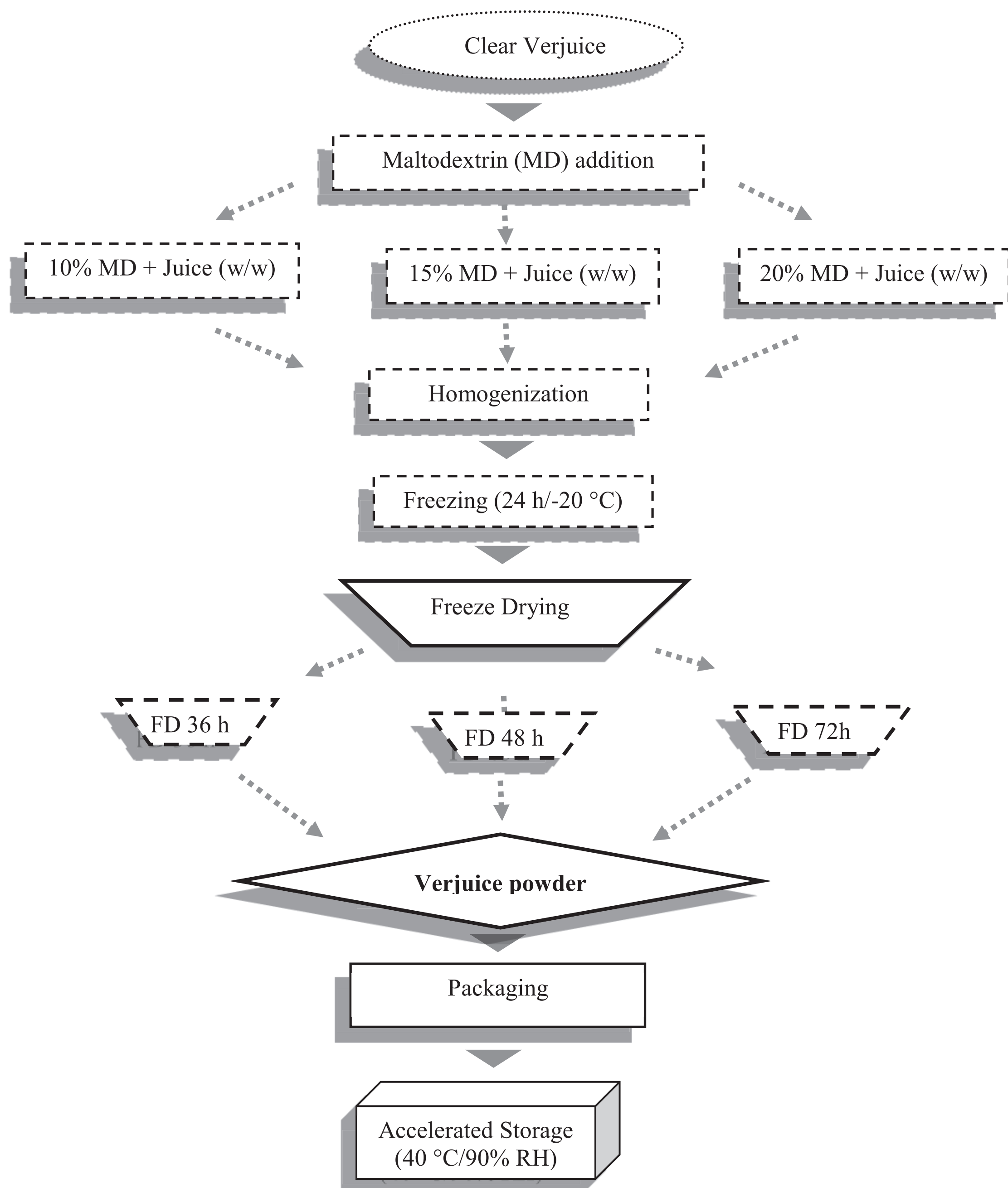


Figure 6.1. The schematic diagram of production and storage of verjuice powder

Table 6.1. Freeze drying experiment conditions

Experiment	Sample code		Maltodextrin concentration in feed mixture (%) (w/w)	Drying time (h)
1	J+10%MD	FD 36h	10	36
2	J+10%MD	FD 48h	10	48
3	J+10%MD	FD 72h	10	72
4	J+15%MD	FD 36h	15	36
5	J+15%MD	FD 48h	15	48
6	J+15%MD	FD 72h	15	72
7	J+20%MD	FD 36h	20	36
8	J+20%MD	FD 48h	20	48
9	J+20%MD	FD 72h	20	72

The yield of freeze drying process was calculated after each process. Several powder properties were measured such as moisture content, water activity, bulk and tapped density, flowability, cohesiveness, solubility, hygroscopicity, glass transition temperature (T_g), color parameters (L*, a*, b*, hue angle, chroma and browning index). Microstructure characteristics of powders were evaluated.

6.1.2. Powder Analyses

6.1.2.1. Product Yield or Product Recovery

The efficiency of the freeze drying process was calculated based on the product yield equation (Equation 6.1) given by Amiri-Rigi, Mohammadifar, Emam-Djomeh, and Mohammadi (2011). The results were expressed as percentage (%).

$$\text{Product recovery or product yield (\%)} = (P * S_p) / (L * S_L) * 100 \qquad (6.1)$$

where P is the weight of powder (g), S_p is the total solid percentage of verjuice powder (%), L is the weight of the feed mixture (verjuice + maltodextrin) (g) and S_L is the total solid percentage of the feed mixture (%).

6.1.2.2. Moisture Content

Moisture contents of the verjuice powders were determined according to AOAC Official Method 934.06 (AOAC, 1990). This method is based on drying of a certain amount of verjuice powder at 70 °C by using a vacuum oven until reaching a constant weight. Results were expressed as percentage (%) or g water in 100 g verjuice powder (g/100 g) and calculated from Equation 6.2.

$$Moisture\ content\ (\%) = \frac{M_1 - M_2}{M_1 - M_0} * 100 \qquad (6.2)$$

where M_0 is the weight of the sample cup (g), M_1 is the weight of the sample cup with powder sample (g) before drying in the oven, M_2 is the weight of the sample cup with powder sample (g) after drying in the oven.

Total dry matter of the verjuice powders was also calculated easily by the equation of "total dry matter (%) = 100 - moisture content (%)".

6.1.2.3. Water Activity

The water activity (a_w) values of verjuice powders were determined using Rotronic HygroLab 3 water activity meter (Rotronic Hygrolab, UK) at room temperature (25 °C). Verjuice powder (3 g) was put into the sample cup that placed inside the sample chamber, and water activity was directly read from the equipment within 2-3 min based on the relative humidity equilibrium between the sample and the headspace of the sample chamber.

6.1.2.4. Bulk and Tapped Density

Bulk density values of verjuice powders were measured according to the method of Goula and Adamopoulos (2005) with some modifications. 2 g of powder sample was placed in 25 mL graduated tube. Bulk density of the powder was calculated by dividing the mass of powder by the volume occupied in the tube. After determination of the bulk density, powder in graduated cylinder was tapped for 120 times and the volume of the

sample was read. Tapped density of the powder was calculated from dividing the mass of powder by the tapped volume in the tube. The measurements were carried out at room temperature (25 °C) and the result was presented as (g/cm^3).

Flowability and cohesiveness values of the powders were assessed in terms of Carr index (CI) (Carr, 1965), and Hausner ratio (HR) (Hausner, 1967), respectively. Both CI, and HR were calculated from the bulk (ρ_{bulk}), and tapped (ρ_{tapped}) densities of the powder by using Equation 6.3 and 6.4 as shown below.

$$CI = \frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} * 100 \tag{6.3}$$

$$HR = \frac{\rho_{tapped}}{\rho_{bulk}} \tag{6.4}$$

6.1.2.5. Solubility

Water solubility index (WSI) of powders was determined using the procedure developed by Eastman and Moore (1984) as adopted by Cano-Chauca et al. (2005). One gram of the verjuice powder (dry basis) was added in 100 mL distilled water and blended by high speed (13000 rpm) for 5 min using a blender (Arçelik K-8020, İstanbul, Turkey). The dispersed powder was then centrifuged at 3000×g for 5 min. A 25 mL aliquot of the supernatant was transferred to a pre-weighed glass petri dish and then oven-dried at 105 °C for 5 h. The solubility of the powder (%) was determined by the weight difference and calculated from Equation 6.5.

$$solubility\ (\%) = \frac{weight\ of\ solids\ in\ 100\ mL\ water\ (solids/25\ mL * 4)}{weight\ of\ sample} * 100 \tag{6.5}$$

6.1.2.6. Hygroscopicity

Hygroscopicity (HG) of freeze-dried verjuice powders were evaluated by modifying the method given in the study of Jaya and Das (2004) and Caparino et al. (2012). 0.5 g of each verjuice powder was spread onto glass sample vessels and placed in a cabinet (TK 120, Nuve, Ankara, Turkey) at a constant temperature (25 °C) and relative

humidity equalized to NaCl saturated solution (76% RH). Powder samples were stored for 7 days in the cabinet and the abilities of powders to adsorb water at the end of 7 days were recorded by gravimetrically. Hygroscopicity or gram amount of adsorbed moisture per 100 gram of dried solids (%) was calculated from the Equation 6.6.

$$HG\ (\%) = \frac{(\Delta m/_{M}) + M_i}{1 + (\Delta m/_{M})} \quad (6.6)$$

where M is the initial weight of verjuice powder (g), M_i is the free water contents of the powder before exposing to the humid air environment (% wb) and Δm is the increase in the weight of the powder after equilibrium (7 days) (g).

6.1.2.7. Color Analyses

Color properties of the verjuice powders were determined by Konica Minolta CR 400 Chromometer (Konica Inc, Japan) using a quartz sample cup. Calibration of the equipment was carried out based on the procedures in section 4.1.5.6 of chapter 4. Verjuice powder (3 g) was placed in the quartz sample cup (50 mL) and results were recorded as average values obtained from three different locations in the powder sample. CIE color parameters, i.e. L* (lightness-darkness), a* (redness-greenness) and b* (yellowness-blueness) values were read directly from the equipment. Chroma (C*), hue angle (h°) and browning index (BI) of the powder samples were calculated with the equations given in section 4.1.5.6 (Equations 4.6, 4.7 and 4.9).

6.1.2.8. Thermal properties

Glass transition temperature (T_g) of verjuice powders were measured by means of a Differential Scanning Calorimeter (DSC) (TA Instruments Q10, New Castle, DE, USA) located in Geothermal Energy Research and Application Centre in Izmir Institute of Technology. The calorimeter was calibrated with indium (156.6 °C) for heat flow and temperature measurements. All measurements were carried out at a heating rate of 10 °C/min, and temperature range varied between -30 and 100 °C. Verjuice powder (6-7 mg) were placed in an aluminium pan (volume of 30 μL) and hermetically sealed by a lid. The

liquid nitrogen at a flow rate of 50 mL/min was used for cooling samples during the experiment. Thermograms were evaluated using TA Universal Analysis 2000 software program.

6.1.2.9. Morphology/Microstructure of Particles

Microstructures or particle morphologies of the verjuice powder were examined by Scanning Electron Microscope (SEM, XL-30S FEG, Philips) located in Materials Research Centre in Izmir Institute of Technology. Small amount of each powder, which were obtained at different MD concentrations (10% MD, 15% MD, 20% MD) and drying times (48 h and 72 h), was placed on the aluminium stubs and coated with a fine layer of gold under high vacuum conditions to provide reflective surface for the electron beam. Inert argon gas under low vacuum was used for gold coating. The pictures of the morphology of the gold-coated powder particles were captured using the scaning electron microscope (SEM) at magnifications of 100x, 300x, 500x and 1000x.

6.1.3. Accelerated Shelf Life Study

The shelf life of a food was defined by UK Institute of Food Science and Technology (IFST) as ‘‘the period of time during which the food product will remain safe, retain its desired sensory, chemical and microbiological characteristics, and comply with any label declaration of nutrition data” (IFST, 1993). Shelf life depends on several environmental factors such as packaging material, light, temperature and humidity of the storage; and intrinsic factors, e.g. composition, acidity, water activity, microbial and oxygen level of the food (Escobedo-Avellaneda, Velazquez, Torres, & Welti-Chanes, 2012; Muzaffar & Kumar, 2017). Food powders are the shelf-stable food products due to having very low water content and their shelf life is very long when stored at room conditions. Accelerated storage studies were carried out under the controlled environment, i.e. at high temperature and humidity in order to avoid the experimental difficulties and to shorten the long-shelf life (Breda, Sanjinez-Argandona, & de AC Correia, 2012; Koc et al., 2010; Muzaffar & Kumar, 2017).

In this study, an accelerated shelf life was carried out with the verjuice powder considering the selected best powder quality characteristics. Freshly squeezed verjuice

was prepared and then maltodextrin was added at a concentration of 20% (w/w). Feed mixture (30 mL) (20.00±0.10 °Brix) was homogenized, pour into falcon tubes (50 mL), frozen, and then dried by using the freeze dryer (-54 °C/0.014 mbar). After, freeze-dried cakes were milled (3000 rpm/10 s) using the grinder. Verjuice powders (5 g) were immediately put into PET/AL/PE packages (Vakumpak Ambalaj, Izmir, Turkey) and heat-sealed to protect powder from moisture and light. The packages were stored in the accelerated conditions described in the study of Muzaffar and Kumar (2017) by using climatic cabinet (TK 120, Nuve, Ankara, Turkey) at a fixed temperature (40 °C) and relative humidity (90% RH).

In order to calculate the accelerated storage period, monolayer and critical moisture content were firstly determined based on the water absorption behavior of verjuice powder at the accelerated conditions of 40 °C/90% RH. Monolayer moisture (bounded water) and critical moisture can be considered as minimum and maximum limits of water content for the physical and chemical stability of powders (Roos, 1995). These values were experimentally calculated for verjuice powder during accelerated storage conditions. The critical water activity corresponding to critical moisture content of verjuice powder was determined using the GAB equation (Equation 6.7) (Devi, Paul, & Sahu, 2016; Muzaffar & Kumar, 2017).

$$a_w = \frac{2+\left(\frac{X_0}{X_{emc}}-1\right)C_g-\left[\left(2+\left(\frac{X_0}{X_{emc}}-1\right)C_g\right)^2-4\left(1-C_g\right)\right]^{0.5}}{2k_g\left(1-C_g\right)} \tag{6.7}$$

where X_0 is the monolayer moisture content (0.0362 kg water /kg dry solid), X_{emc} is the equilibrium (critical) moisture content (0.0532 kg water /kg dry solid), C_g (8.183) and k_g (0.962) are GAB model constants. GAB model constants (C_g and k_g) were directly taken from the study of Muzaffar and Kumar (2017) conducted with the tamarind juice powder having the acidity content (9.5%) similar to verjuice powder (11%).

The accelerated shelf life of verjuice powder was then predicted using the shelf life equation (Equation 6.8) given in the study of Devi et al. (2016):

$$\theta = \frac{X_s*(X_c-X_i)}{k*\,A_p*(Rh\,p*-a_w\,p)} \tag{6.8}$$

where X_s is the dry weight of powder inside the package (0.0043 kg dry solid), X_c is the critical moisture content (0.0532 kg water/kg dry solid), X_i is the initial moisture content (0.0356 kg water/kg dry solid), k is the permeability of the PET/AL/PE packages ($1.77*10^{-8}$ kg/m^2*day*Pa) measured by a permeability equipment (Permatran-W-3/33, Mocon Inc., Minneapolis, MN, USA) in Chemical Engineering Department in Izmir Institute of Technology, A_p is the surface area of the PET/AL/PE packages (0.0176 m^2), p* is the saturated water vapour pressure (7289 Pa) at the storage temperature (40 °C), Rh is the relative humidity of the storage cabinet (0.90), a_w is the critical water activity (0.43) (calculated from equation 6.7) of the powder at the storage temperature. According to Equation 6.8, the accelerated shelf life (θ) of verjuice powder was predicted as approximately 70 days.

The verjuice powder samples stored at the temperature of 40 °C and relative humidity of 90% RH were monitored during 70 days with a two-week interval (0th, 14th, 28th, 42nd, 56th, 70th day). Moisture content, water activity, several powder properties (bulk and tapped density, flowability, cohesiveness) and color parameters (L*, a*, b*, C*, h°, ΔE, BI) were analysed during the accelerated shelf life. Experiments were repeated two times.

6.1.4. Statistical Analysis

One-way analysis of variance (ANOVA) were carried out for drying processes to determine how significantly the independent variables affect the dependent variables. Independent variables were different maltodextrin concentrations (10%, 15% and 20% MD [w/w]) and freeze drying times (36, 48 and 72 h) used in the verjuice powder production. Dependent variables were quality properties of verjuice powder (drying yield, moisture content, a_w, bulk and tapped density, flowability, cohesiveness, solubility, hygroscopicity, T_g, color parameters). All results were given with their means and standard deviations. Least significant difference (LSD) test was applied and the means of data were compared by Tukey's pairwise comparison test. Minitab 16 software program (Minitab Inc., State College, PA, USA) was employed for one-way ANOVA and least significant difference test of the results. All FD process experiments were repeated three times and the measurements in the shelf life study were repeated two times.

6.2. Results and Discussion

6.2.1. Product Yield of the Freeze Drying Process

Verjuice powders obtained under different processing conditions of freeze drying were produced with high yields (>95%) (Figure 6.2). The product yield in freeze drying slightly increased when the concentration of maltodextrin in the feed mixture was increased. The lowest and the highest yield was found as 95.25±2.73% and 98.76±0.61% for the powders containing MD at a concentration of 10% and 20%, respectively, after freeze drying of verjuice for 36 h (FD 36h) and 72 h (FD 72h). The reason of this difference could be attributed to the maltodextrin used as a carrier agent in the juice for prevention of powders from adhering to the wall of the tube during drying. Thus, stickiness of the powder was reduced by increasing maltodextrin concentration (Caliskan & Dirim, 2016; Jafari, Ghalenoei, & Dehnad, 2017; Oberoi & Sogi, 2015). However, the product yields of all samples produced at different drying conditions (Figure 6.2) were not statistically different from each other ($p>0.05$).

In this study, freeze drying conditions of FD 48h and FD 72h were very efficient for drying of verjuice containing 15% and 20% of maltodextrin by achieving approximately higher than 98% of product yield. Caliskan and Dirim (2016) was also reported that the sumac extract powders did not lose their solid content by freeze drying technique. As a result, their process yield was calculated as 100%. Baeghbali, Niakousari, and Farahnaky (2016) compared the yield of spray drying and freeze drying processes for the pomegranate juice powder containing Gum Arabic (35% db) as a carrier agent. They found that, the yield was significantly higher in the freeze drying (96.7%) compared to the spray drying (78.1%). Similary, Wilkowska, Ambroziak, Czyzowska, and Adamiec (2016) observed higher yield for freeze-dried blueberry juice powder (78.1%) compared to spray-dried samples (43.9%). This was attributed to the powder loss caused by sticking of dried particles on the wall of the spray drier and cyclone. In another study, the drying yield for the feed mixture of cactus pear juice (juice:MD, 5:1 [w/w]) was found to be higher in freeze drying than that of spray drying. (Moßhammer, Stintzing, & Carle, 2006).

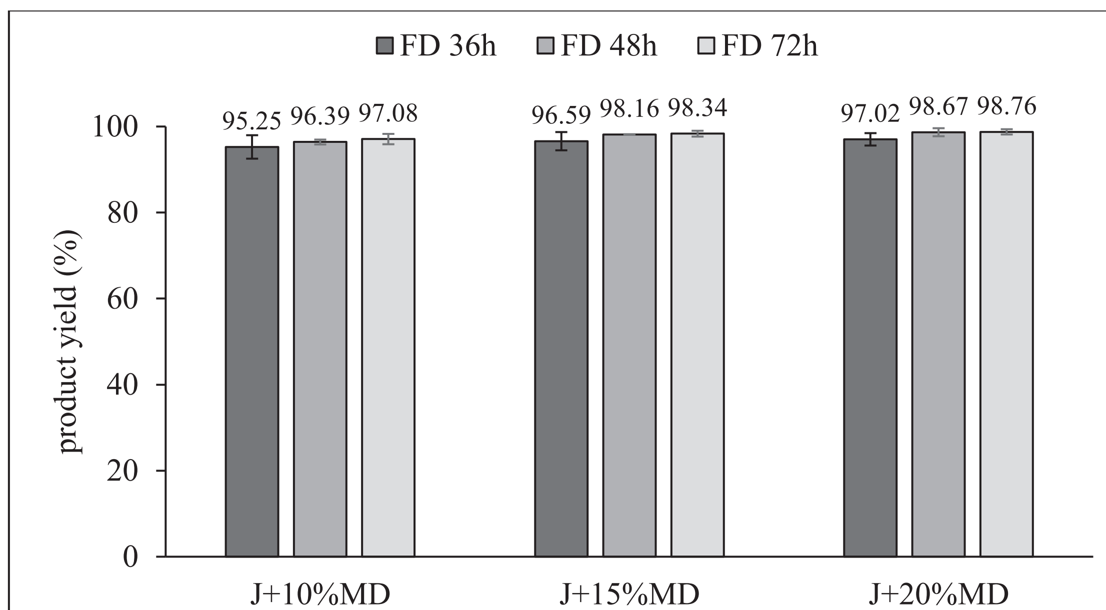


Figure 6.2. Product yield (%) of freeze drying for verjuice powders
(J: verjuice, MD: Maltodextrin, FD: freeze drying)

6.2.2. Moisture Content and Water Activity of Verjuice Powders

The moisture content (%) and water activity (a_w) of the verjuice powders after freeze drying (FD) for 36 h, 48 h and 72 h were listed in Table 6.2. According to table, moisture contents and a_w of the verjuice powders were ranged between 3.02-8.85% and 0.15-0.50, respectively. Oberoi and Sogi (2015) used 10% MD concentration as a carrier in the production of freeze-dried watermelon powder. The moisture content (4.43-5.86% db) and a_w (0.40-0.44) of freeze-dried watermelon powder in their study were in agreement with the results of the freeze-dried verjuice powder (48h and 72 h). Moisture content and water activity of the verjuice powders were significantly decreased by increasing drying time from 36 h to 72 h for all MD concentration ($p \leq 0.05$). Similarly, higher MD concentration caused a decrease in the moisture content and water activity of verjuice powders during freeze drying process. This was because, the feed mixture before drying process had higher solid content or less free water content for evaporation when the higher concentration level of maltodextrin was added to the juice (Oberoi & Sogi, 2015; Quek et al., 2007). Therefore, the moisture content of the freeze-dried powders reduced after freeze drying with the higher amount of maltodextrin in the feed. Similarly, Oberoi and Sogi (2015) found that moisture content of freeze-dried watermelon powder significantly decreased by increasing MD concentration from 3% to 10% (w/w). Kha et

al. (2010) found that moisture content of Gac fruit juice powder decreased significantly from 4.87 to 4.06% by the increment of maltodextrin concentration in the feed mixture from 10% to 20% (w/v). There were other studies reported that the increasing MD concentration resulted in less moisture content and water activity in powders (Islam, Kitamura, Yamano, & Kitamura, 2016; Quek et al., 2007).

Table 6.2. Moisture content and water activity of verjuice powders after freeze drying

	Feed mixture	FD 36h	FD 48h	FD 72h
Moisture (%)	J + 10%MD	8.85 ±0.18a	6.24 ±0.15c	4.12 ±0.15de
	J + 15%MD	8.82 ±0.13a	4.54 ±0.10d	3.76 ±0.35e
	J + 20%MD	7.11 ±0.33b	3.98 ±0.11de	3.02 ±0.30f
a_w	J + 10%MD	0.46 ±0.03*ab*	0.40 ±0.01*bc*	0.33 ±0.03*cd*
	J + 15%MD	0.50 ±0.01*a*	0.25 ±0.03*de*	0.23 ±0.05*ef*
	J + 20%MD	0.47 ±0.02*ab*	0.18 ±0.02*ef*	0.15 ±0.06*f*

J: verjuice, MD: Maltodextrin, FD: freeze drying. Different small letters indicate differences between samples based on One-way ANOVA analyses compared by Tukey comparison test ($p \leq 0.05$). Normal letter (a, b, c, d, e, f): differences between moisture content, Italic letter (*a, b, c, d, e, f*): differences between a_w.

Drying time and carrier concentration were crucial parameters to obtain a stable verjuice powder. The studies cited in the literature generally indicated that, the food powders should have less than 4-5% moisture content and lower than 0.2-0.25 water activity in order to have a long shelf life (Koc et al., 2010; Kumar & Mishra, 2004). The polymerization degree (DE) of maltodextrin could be another important factor for the moisture of the powders. It was reported that high polymerization degree of maltodextrin (20 DE), used as a carrier in the drying of cactus pear juice, produced the powders having lower moisture content comparing to low polymerization degree (10 DE) (Rodriguez-Hernandez, Gonzalez-Garcia, Grajales-Lagunes, Ruiz-Cabrera, & Abud-Archila, 2005). In this study, maltodextrin of 20 DE was used in order to produce verjuice powder with low moisture content.

Verjuice feed mixtures having different maltodextrin concentrations were freeze-dried for 36 h (FD 36h) (Table 6.2). It was found that powders had higher moisture content and a_w, exhibiting unstable powder properties. Besides, it was observed that these powder samples immediately became very sticky and other physical powder analyses (bulk density, tapped density, flowability and cohesiveness) could not be done. Thus,

freeze-dried verjuice powders for 48 h and 72 h (FD 48h and FD 72h) were analysed only. Moisture content (6.24%) and water activity (0.40) of the verjuice powder having 10% MD after FD 48h was also above the limits and physical powder properties of this product was investigated in more detail in the next section.

6.2.3. Physical Properties of Verjuice Powder

Physical properties of verjuice powder (bulk density, tapped density, flowability, cohesiveness, solubility and hygroscopicity) obtained after 48 h (FD 48h) and 72 h (FD 72h) freeze drying processes were listed in Table 6.3.

Bulk and Tapped Density

According to Table 6.3, bulk and tapped densities of verjuice powders obtained from the feed mixtures having 10%, 15% and 20% MD concentration subjected to FD 48h and FD 72h processes were not significantly different ($p>0.05$). Therefore, concentration of maltodextrin was not a distinctive factor for each of 48 h and 72 h freeze drying processes. However, bulk and tapped densities of powders obtained after FD 72h were slightly increased. The bulk density of verjuice powder containing 20% MD concentration obtained by drying for 48 h (FD 48) was significantly lower (0.32 g/cm^3) than the one subjected to drying for 72 h (FD 72h) (0.47 g/cm^3) ($p\leq0.05$). It was reported that there was a correlation between moisture content, MD concentration and bulk density of the powder (Abadio et al., 2004; Fazaeli et al., 2012; Goula & Adamopoulos, 2005; Jafari et al., 2017). Goula and Adamopoulos (2005) indicated that powder having a higher moisture content became stickier and resulted in higher bulk volume by the increase of particles interspace. As a result, bulk density of powder was low. Abadio et al. (2004), Fazaeli et al. (2012) and Jafari et al. (2017) studied the relationship between maltodextrin concentration in the feed mixture and bulk density of the powder. They observed that the higher MD concentration caused to lower the bulk density of powder by reducing its moisture content.

Table 6.3. Physical properties of verjuice powders after freeze drying

	FD 48h			FD 72h		
	J + 10%MD	**J + 15%MD**	**J + 20%MD**	**J + 10%MD**	**J + 15%MD**	**J + 20%MD**
Bulk density (g/cm^3)	0.37 ±0.04abc	0.33 ±0.03bc	0.32 ±0.03c	0.52 ±0.08abd	0.46 ±0.03bd	0.47 ±0.04d
Tapped density (g/cm^3)	0.49 ±0.02ab	0.45 ±0.03ab	0.39 ±0.02b	0.67 ±0.11c	0.56 ±0.06ac	0.59 ±0.05ac
Flowability (CI)	28.30 ±0.82a	21.46 ±0.70b	20.48 ±0.82b	21.75 ±0.96b	21.38 ±0.50b	20.42 ±0.81b
Cohesiveness (HR)	1.35 ±0.03a	1.27 ±0.01b	1.26 ±0.01b	1.28 ±0.02b	1.27 ±0.01b	1.24 ±0.02b
Solubility (%)	88.29 ±0.53a	91.91 ±0.35b	95.88 ±0.42c	88.43 ±0.59a	91.37 ±0.85b	96.04 ±0.91c
Hygroscopicity (%)	17.41 ±0.83a	16.09 ±0.61bc	15.12 ±0.21c	17.10 ±0.26ab	15.63 ±0.15c	15.08 ±0.17c

J: verjuice, MD: Maltodextrin, FD: freeze drying. Different small letters in the same line indicate differencies between samples based on One way ANOVA analyses compared by Tukey test ($p \leq 0.05$).

Higher MD concentration, as a coating agent (skin-forming effect on the particles), could lead to larger air-trapped particles. For this reason, the bulk density was reduced with a larger volume of particles. In another study, Franceschinis et al. (2014) noted that crushing conditions of freeze-dried cakes, which were related with particle morphology, were crucial as well for bulk density of powders. It was also reported that the inter-particle spaces could be reduced with the smaller size of particles, thus, bulk density was increased (Caparino et al., 2012). In this study, verjuice powder obtained from FD 48h had less bulk density with a higher moisture content and bulk volume. The bulk and tapped density of verjuice powders did not significantly change by MD concentration ($p>0.05$). However, a slight decrease was observed in the verjuice powders obtained from the feed mixture having 20% MD with a lower moisture content (Table 6.2) compared to the feed having 10% MD. Similarly, Caliskan and Dirim (2016) found that the bulk and tapped density of freeze-dried sumac extract powder did not considerably vary with the different MD concentrations (20, 25 and 30% MD) in the feed mixture. All bulk densities of verjuice powders obtained in this study (Table 6.3) were within the desired limits which were between 0.3 and 0.6 g/cm^3 (Erbay, Koca, Kaymak-Ertekin, & Ucuncu, 2015; Vardin & Yasar, 2012).

Flowability and Cohesiveness

Carr index (CI) and Hausner ratio (HR) calculated from the bulk and tapped densities were responsible for flowability and cohesiveness of powder and listed in Table 6.3. Jinapong et al. (2008) were classified the flowability (CI) as very good (< 15), good (15-20), fair (20-35), bad (35-45), and very bad (>45); while the cohesiveness (HR) as low (< 1.2), intermediate (1.2-1.4) and high (> 1.4). Carr Index of verjuice powders were statistically similar ($p>0.05$) and they were in the range of 20.42 and 21.75 except the one containing 10% MD obtained in the process of FD 48h (28.30). These values (except the feed mixture having 10% MD dried for 48 h) were considered to be in the good flowability class according to Jinapong et al. (2008). Verjuice powder exhibited good flowability property by increasing the amount of MD in the verjuice. The cohesivess of the all verjuice powders were in the range of intermediate level (between 1.2 and 1.4) according to the classification of hausner ratio cited in Jinapong et al. (2008). However, verjuice powder having the 10% MD subjected to FD 48h process was found to be the

most cohesive ($p \leq 0.05$) with the highest Haustner ratio (1.35). Caliskan and Dirim (2016) reported that sumac extract powders with a higher MD concentration (25%) resulted in good flowability (19.42) and low cohesiveness (1.24) compared to the ones with lower MD concentration. High flowability and low cohesiveness of powders might be related to their moisture content. It was reported that powder produced by adding a higher amount of maltodextrin to a feed mixture, had a lower moisture content, and yielded higher flowability and lower cohesiveness properties (Fitzpatrick et al., 2007). The best flowable and the least cohesive powders were produced from verjuice having the highest maltodextrin concentration subjected to FD 48h and FD 72h (Table 6.3).

Solubility

Solubility is an important parameter for reconstitution of the powders and its functional usage; good powders are required to have higher solubility (Barbosa-Canovas et al., 2005a; Erbay et al., 2015). Solubility of the verjuice powders were observed in a range of 88.29% and 96.04% (Table 6.3). It was noticed that verjuice powders showed similar solubility when the same MD concentration levels were used for each drying time (FD 48h and FD 72h) (Figure 6.3). However, solubility of powders was increased significantly by increasing the MD concentration ($p \leq 0.05$). The reason of high solubility of powders could be connected to the maltodextrin showing high solubility characteristics in water (Cano-Chauca et al., 2005; Caparino et al., 2012; Goula & Adamopoulos, 2010). Besides, verjuice powders having higher bulk density showed lower solubility (Table 6.3). These results were in agreement with the literature reporting an inverse relationship between water solubility and bulk density of the powder (Fazaeli et al., 2012). Besides, solubility could be related to the water content. Caparino et al. (2012) reported 89.7% solubility for freeze-dried mango powder with a water content of 2.3%, whereas freeze-dried verjuice powder having 3% water had a better solubility value (96.04%). Baeghbali et al. (2016) measured 89.1% solubility in freeze-dried pomegranate juice powder having 8.5% moisture content. However, a slightly lower solubility (88.29%) was obtained in the verjuice powder having 6.24% moisture content.

Hygroscopicity

Hygroscopicity (HG), which is defined as the ability of powders to keep moisture in their structure, is one of the important parameters for the shelf life of powder products (Jaya & Das, 2004; Rodriguez-Hernandez et al., 2005). Hygroscopicity of verjuice powders was noticeably higher in the lower MD concentration (Table 6.3). It was measured as 17.41% (FD 48h) and 17.10% (FD 72h) after drying of the feed mixture having 10% MD. However, these values were 15.12% (FD 48h) and 15.08% (FD 72h) in powders prepared from the feed mixtures with 20% MD. Therefore, it was concluded that verjuice powders became less hygroscopic and more stable by increasing amount of MD in feed mixtures. These values were found to be lower compared to the hygroscopicity of freeze-dried mango powder (HG: 18%) reported by Caparino et al. (2012). Islam et al. (2016) indicated that hygroscopicity of orange juice powder significantly decreased by increasing juice:maltodextrin ratio from 50:50 (HG: 19.5%) to 30:70 (HG: 14.3%). In this study, by increasing MD concentration, hygroscopicity and solubility of the verjuice powders were inversely related, i.e. less hygroscopic powders were more soluble in the water (Figure 6.3). This inverse correlation was in agreement with the study of Islam et al. (2016). Similarly, pomegranate juice powders obtained by Vardin and Yasar (2012) and acai juice powders obtained by Tonon, Brabet, and Hubinger (2008) were reported to be less hygroscopic when higher levels of MD were used.

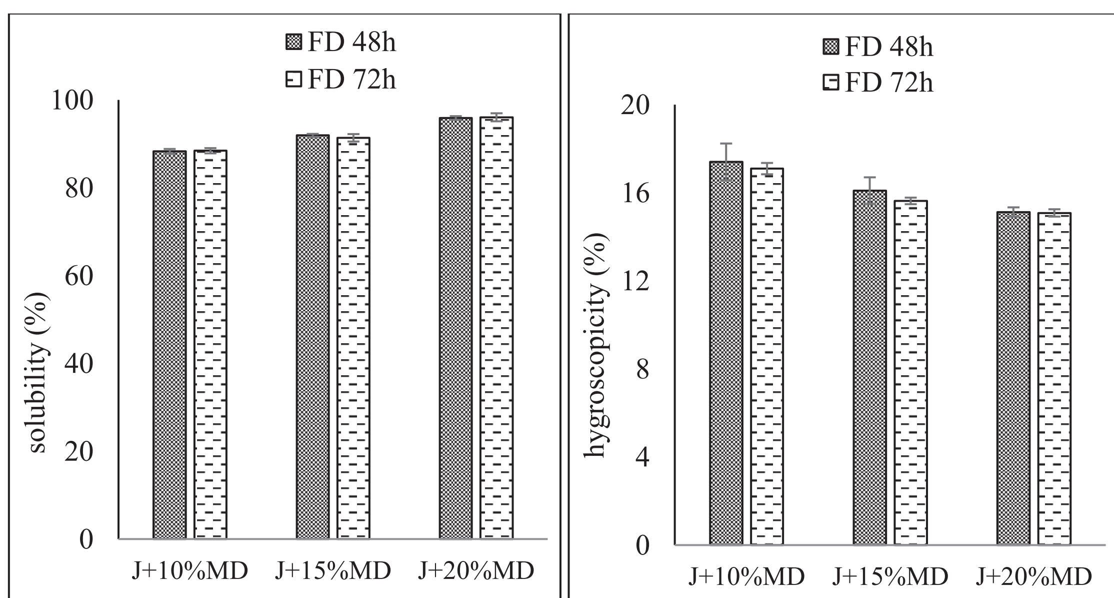


Figure 6.3. Solubility and hygroscopicity of verjuice powders after freeze drying
(J: verjuice, MD: Maltodextrin, FD: freeze drying)

6.2.4. Color Analyses of Verjuice Powders

Drying time did not have an important effect on the color parameters of verjuice powders. However, there were significant variations between powders containing maltodextrin at different concentrations (Table 6.4 and Figure 6.4). L* (lightness-darkness) values of the verjuice powders were considerably increased from 82.54 to 88.79 for FD 48h, and from 80.92 to 87.94 for FD 72h processes ($p \leq 0.05$). The changes of lightness in the powders could be the result of the increase of maltodextrin in the powder product. Caliskan and Dirim (2016), Franceschinis et al. (2014) and Kha et al. (2010) reported that the maltodextrin has a white color (high L* value) and using higher concentration of maltodextrin in a feed mixture may be the reason of an increase in L* value of the powders. Kha et al. (2010) observed that L* value of the spray-dried Gac aril powder obtained from the feed having 20% MD (L*: approx. 85) was significantly higher than the powder obtained from the feed having 10% MD (L*: approx. 80). Besides, the lightness of the verjuice powder was well-protected due to the fact that freeze drying process was applied at relatively low temperature. Caliskan and Dirim (2016) reported that L* value of spray-dried sumac extract powders were lower than the freeze-dried ones. Because, the powders lost their color by the effect of high temperature. Increase of MD level in the feed mixture from 10% to 20% led to a remarkable decrease in a* (redness-greenness) values of powders, i.e. the redness values of the verjuice powders having 10% MD (1.31 and 1.74) were measured significantly higher than the powders having 20% MD (0.48 and 0.65) ($p \leq 0.05$) (Table 6.4 and Figure 6.4). Considering b*(yellowness-blueness) values, the verjuice powders obtained from the feed having 10% MD were distinguishably more yellow (b*: 17.24-18.41) than the other powders (b*: 14.63-11.06). Similarly, Caliskan and Dirim (2016) and Oberoi and Sogi (2015) reported low a* and b* values by the addition of maltodextrin after freeze drying. They were also reported that color of the powder products was protected better in freeze drying in comparison to spray drying. Telis and Martinez-Navarrete (2010) indicated that there was a relationship between water activity and color properties of the powders. They found that the freeze-dried grapefruit powder having higher water activity had lower L*, higher a* and b* values. In this study, the verjuice powders having lower L*, higher a* and b* values were obtained from the powders with higher water activity, in agreement with the literature. Hue angles of the powders were measured between 83.43° and 87.83° for all process

conditions (Table 6.4 and Figure 6.4). In this study, due to the dominated yellow color of verjuice, yellow powders (around 90° hue angle) were obtained based on the color scale given by Pathare et al. (2013). Quek et al. (2007) indicated that hue angle of the powder was influenced by the degradation of pigments at high temperature drying processes. Therefore, hue angle of the verjuice powders could be expected to be not changed after freeze drying. Similarly, it was also reported that hue angle of the freeze-dried powders did not change at different water activity levels (Telis & Martinez-Navarrete, 2010). Chroma value (C*) is responsible for the color intensity or saturation of powder. Lower chroma value was detected in the powders which had higher maltodextrin concentration. Consequently, it was found that maltodextrin affected the color intensity of verjuice powders, i.e. less saturated powder was obtained when the higher MD concentration was used in the feed mixture. Similarly, Oberoi and Sogi (2015) detected lower chroma values in freeze-dried watermelon juice powder upon increasing the MD concentration from 3% to 10%. The results of this study were consistent with other previous studies (Kha et al., 2010; Quek et al., 2007).

It was generally reported that freeze drying process causes less browning in powders compared to other drying processes (Krokida, Maroulis, & Saravacos, 2001; Que, et al., 2008). Krokida et al. (2001) indicated that freeze drying process at low temperature prevents the powders from color degradation and browning reactions providing a stable L*, a* and b* values. It was also reported that freeze drying is the most suitable drying method avoiding the degredation of carotenoids and undesirable pigment formation (Topuz, Feng, & Kushad, 2009). In this study, browning index (BI) of verjuice powders were significantly decreased with increasing maltodextrin concentration (Table 6.4 and Figure 6.4). However, drying time did not have profound effect on the browning of verjuice powders. BI value of verjuice powder obtained from the feed with 10% MD was in the range of 24.72 and 27.16. However, this value was decreased significantly to the range of 12.70 and 14.45 for verjuice powder obtained from the feed with 20% MD. The reason of this could be attributed to concentration used maltodextrin as a coating agent in this study. Because, maltodextrin protected powders from oxygen and light which were the main causes of enzymatic and chemical reactions (Wang, Zhaoxin, Fengxia, & Xiaomei, 2009). It was also reported that the decrease of browning index of the powder was a result of increasing of the carrier concentration, i.e., higher L* value, lower a* and b* values were resulted in lower BI value in powders (Fazaeli, Emam-Djomeh, Omid, & Kalbasi-Ashtari, 2013a).

Table 6.4. Color properties of verjuice powders after freeze drying

	FD 48h			FD 72h		
	J + 10%MD	**J + 15%MD**	**J + 20%MD**	**J + 10%MD**	**J + 15%MD**	**J + 20%MD**
L*	82.54 ±0.35a	87.22 ±0.57b	88.79 ±0.22c	80.92 ±0.34d	87.14 ±0.53b	87.94 ±0.30bc
a*	1.31 ±0.17ab	1.09 ±0.15ab	0.48 ±0.13a	1.74 ±0.73b	0.74 ±0.16a	0.65 ±0.11a
b*	17.24 ±0.53a	14.63 ±0.25b	13.45 ±0.44bc	18.41 ±2.06a	13.80 ±0.50bc	11.66 ±0.13c
Chroma	17.38 ±0.47a	14.69 ±0.23b	9.74 ±0.62c	18.54 ±2.14a	13.82 ±0.51b	11.68 ±0.13bc
Hue angle	85.56 ±0.50ab	87.72 ±0.71a	87.83 ±1.38a	83.43 ±1.81b	86.93 ±0.58a	86.81 ±0.48a
BI	24.72 ±1.00a	19.54 ±0.56b	12.70 ±0.93c	27.16 ±3.96a	17.49 ±0.89b	14.45 ±0.30c

J: verjuice, MD: Maltodextrin, FD: freeze drying. Different small letters in the same line indicate differencies between samples based on One way ANOVA analyses compared by Tukey test ($p \leq 0.05$).

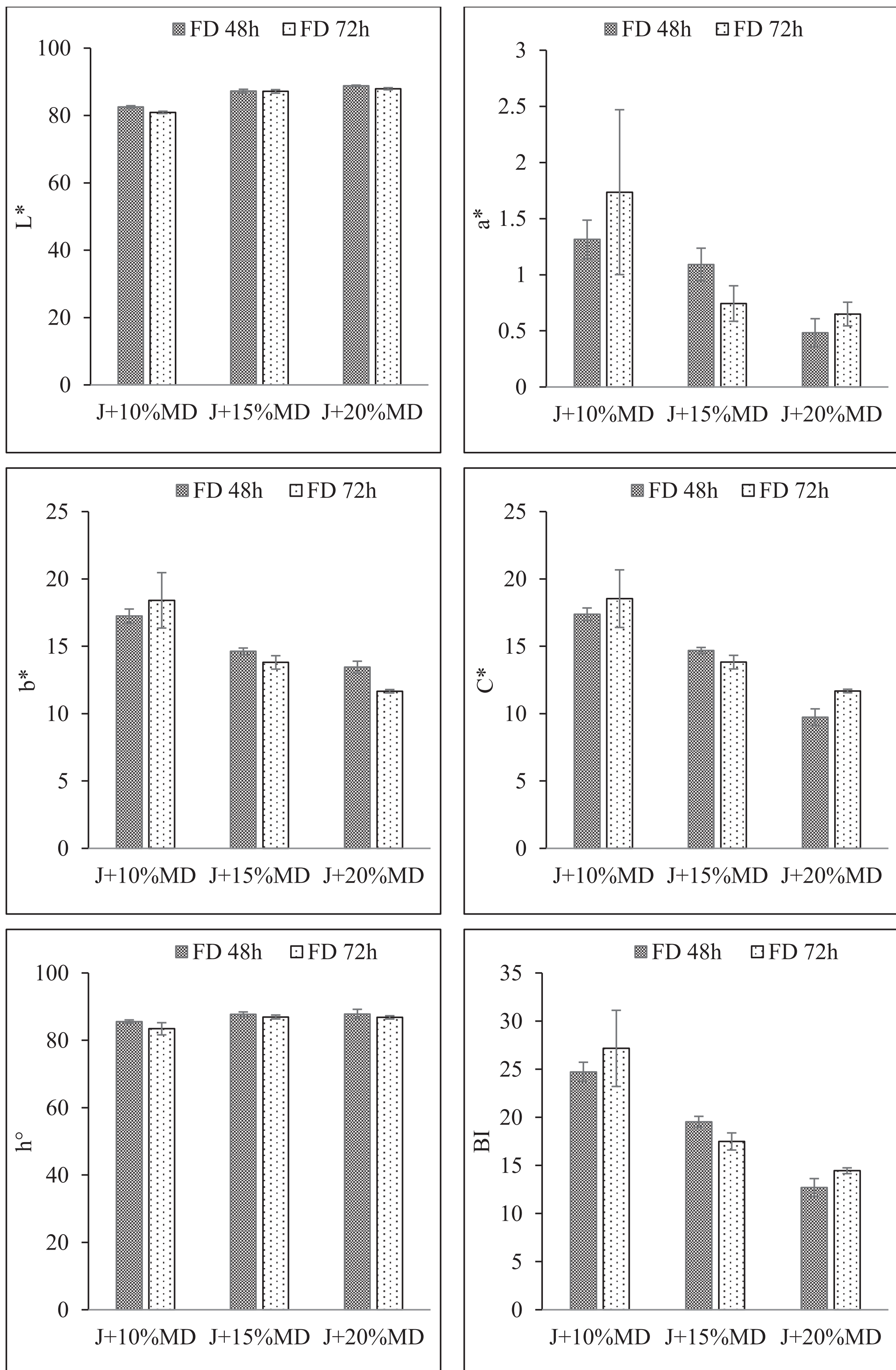


Figure 6.4. The change in color parameters of verjuice powder after freeze drying
(J: verjuice, MD: Maltodextrin, FD: freeze drying. C*: chroma, h°: hue angle, BI: browning index)

6.2.5. Thermal Properties of Verjuice Powders

The amorphous materials such as carbohydrates in foods could be in the glassy or (highly viscous or solid-like) or rubbery (liquid-like) form depending on the variations of temperature and moisture content. These materials are very stable in glassy state at low temperatures. However, they may converted to the unstable rubbery state by the increase of temperature or water content in foods. Therefore, the glass transition temperature (T_g) is very important for the stability of the amorphous foods because the viscous or solid form of these materials can become rubbery or liquid form at this temperature (Fongin et al., 2017; Roos, 1995; Silva, Sobral, & Kieckbusch, 2006; Telis & Sobral, 2002). T_g of the all verjuice powders obtained from the feed mixture having different MD concentrations and drying times were given in Figure 6.5. In addition, all data ($T_{g\ onset}$, T_g and $T_{g\ endpoint}$) and DSC diagrams were presented in Appendix C (Table C.1 and Figure C.1). According to the Figure 6.5, it was clearly seen that the higher the drying time and maltodextrin concentration, the higher the T_g value in powders. Maltodextrin has a high molecular weight and it was used in the drying of sugar based products in order to increase T_g values and avoid stickness of powders (Caparino et al., 2012; Jaya & Das, 2004). The lowest Tg value (12.74 °C) was measured in the powder produced by freeze drying of the feed mixture having low amount of maltodextrin (10% MD) which was dried for 48 h (FD 48h). However, T_g of the powders produced with 20% MD had the highest values for both drying times, i.e. FD 48h (51.98 °C) and FD 72 h (51.54 °C). Therefore, it can be concluded that these verjuice powders will not be sticky if they are stored at room temperature (20 °C). It is generally reported that if the storage temperature of powder is 20 °C higher than T_g value of the powder, it will become sticky (Bhandari, Datta, & Howes, 1997). Besides, it is speculated that powders having higher T_g (20% MD, FD 48 & FD 72h) will be more stable and have a longer shelf life if stored at room temperature conditions.

Moisture content can also affect T_g value of powders. Zhao, Liu, Wen, Xiao, and Ni (2015) reported that water has a plasticizing effect on the amorphous structure of the powder. Temperature (T_g) from the viscous to the rubbery state decreased upon increasing water content. Drying time did not change T_g of powders produced with 15% and 20% maltodextrin content. However, there was a noticeable change in T_g value of powders obtained with 10% MD (Figure 6.5). This could be attributed to the higher water content.

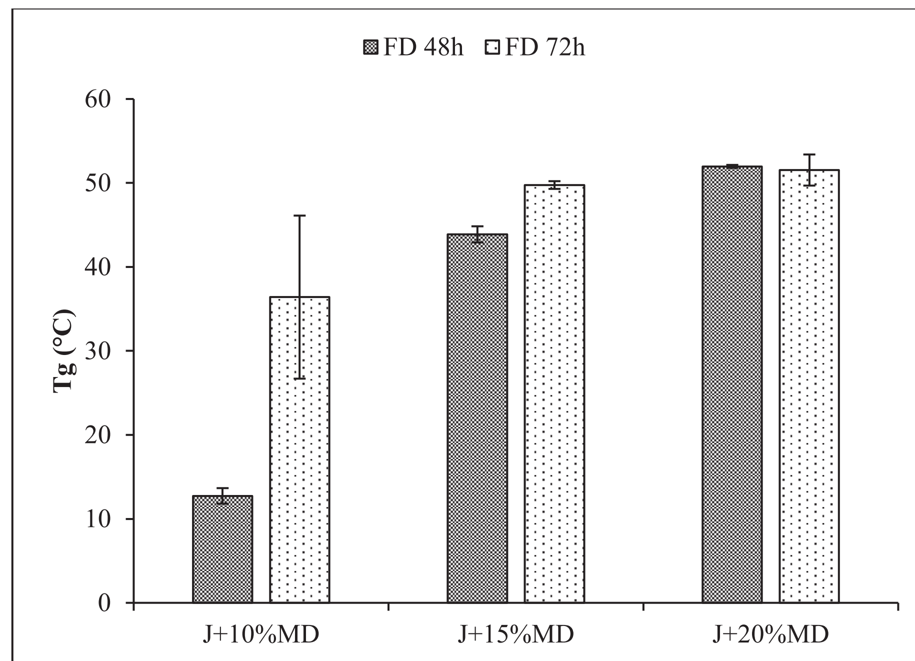


Figure 6.5. Glass transition temperature (T_g) of verjuice powders after freeze drying
(J: verjuice, MD: Maltodextrin, FD: freeze drying)

6.2.6. Microstructure of Verjuice Powders

Scanning electron micrographs obtained at the magnifications of 100x and 1000x were depicted in Figure 6.6 and Figure 6.7, respectively. Micrographs of the verjuice powders indicated that the size of the powder particles became smaller by increasing drying time from 48 h to 72 h, and maltodextrin concentration from 10% to 20% in the verjuice. It was reported that high molecular weight of a carrier could be effective on the size of particles, i.e. smaller particles were obtained by adding the higher concentration of maltodextrin to the feed (Harnkarnsujarit, Charoenrein, & Roos, 2012). Besides, particles appeared irregularly shaped after freeze drying process. Micrographs obtained in this study was similar to the micrographs in the study of Caparino et al. (2012). They observed skeletal-like and porous structure in the freeze-dried mango powder. It was reported that the removal of the ice in the frozen feeding solution during freeze drying protected the shape of the product without any volume loss (Ratti, 2001). Thus, freeze-dried powders looked like more porous comparing to the other samples obtained by different drying techniques. However, Caparino et al. (2012) found that the particle porosity decreased by reducing the size of particles. In this study, smaller size of particles had smoother shape at a magnification of 1000x (Figure 6.7).

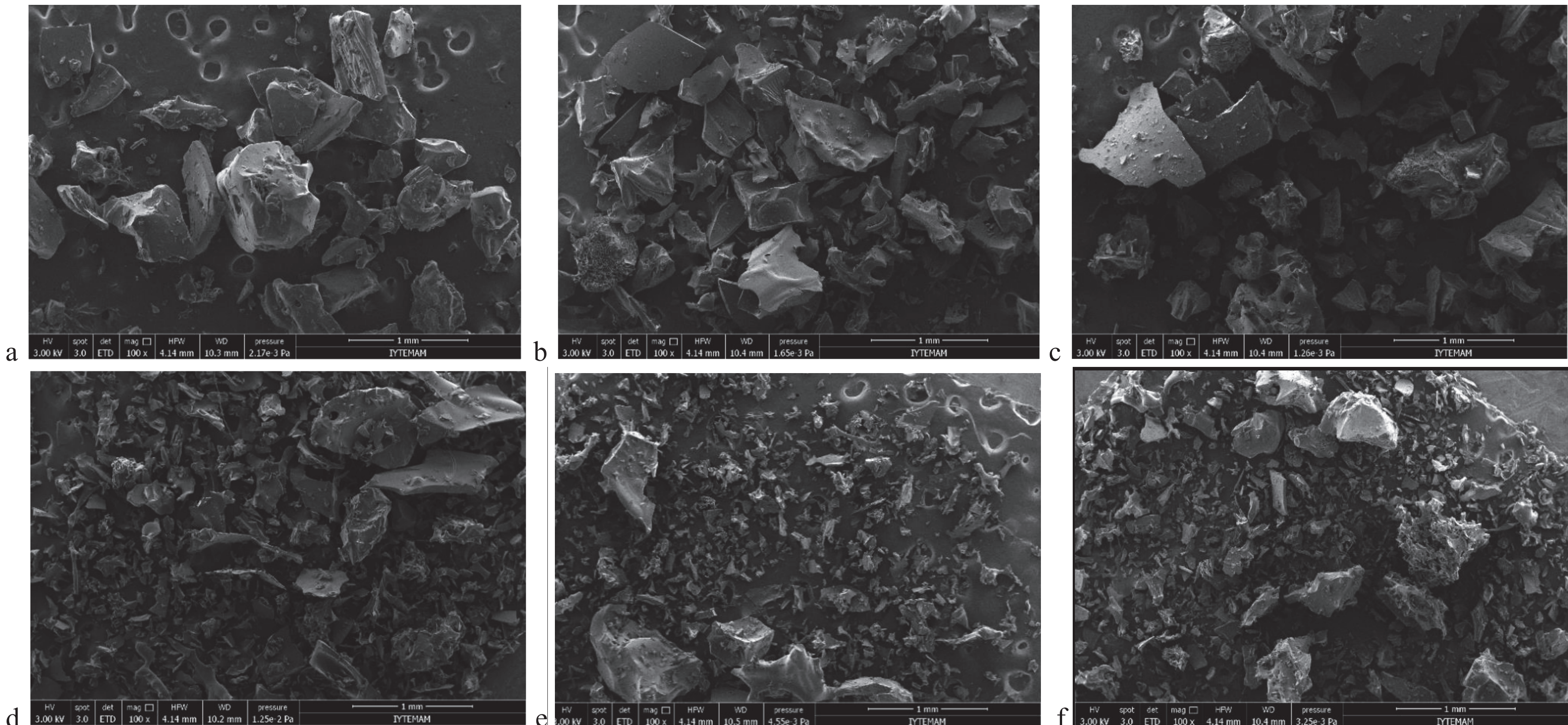
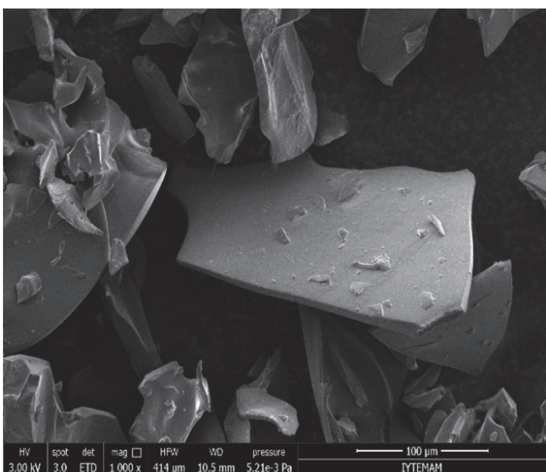
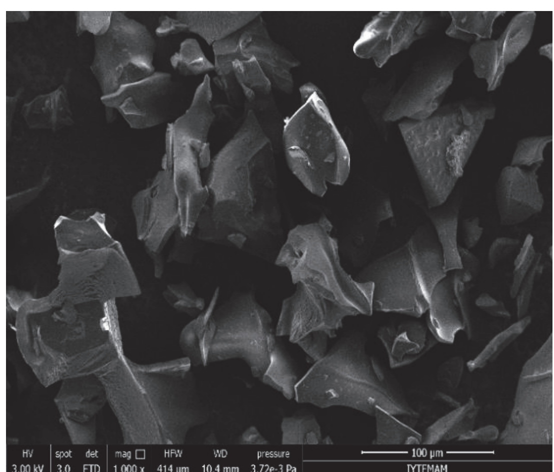


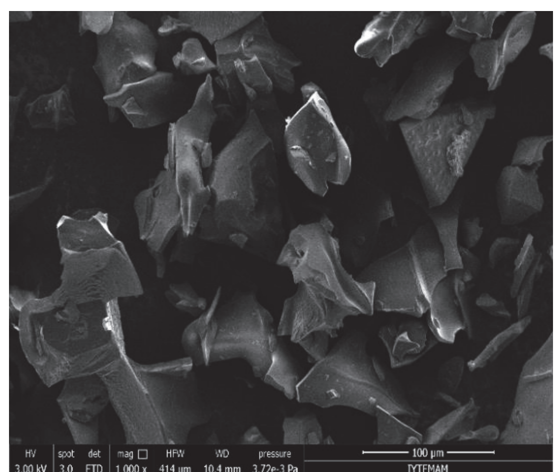
Figure 6.6. Scanning Electron Micrographs of verjuice powder after freeze drying for 48h and 72h (magnification at 100x) (48h freeze-dried powder containing a: MD10%, b: MD15%, c: MD20%; 72h freeze-dried powder containing d: MD10%, e: MD15%, f: MD20%)



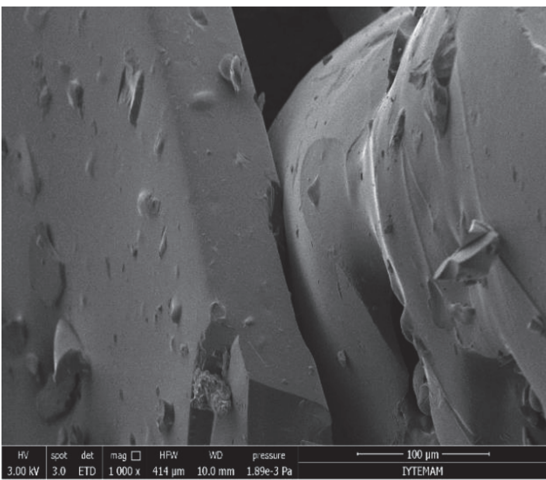
A



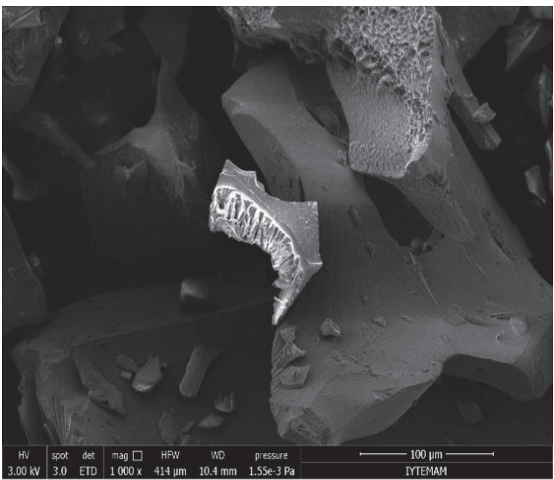
B



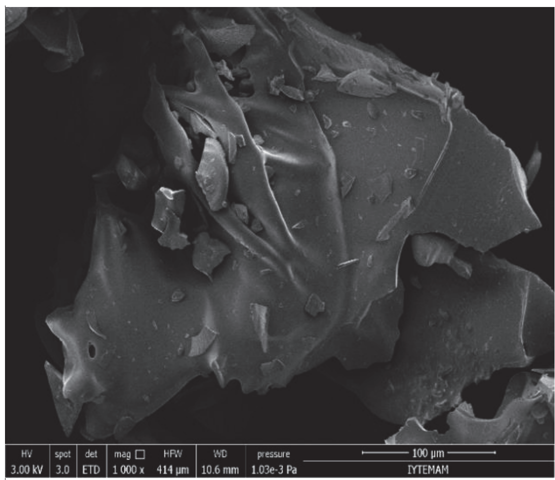
C



D



E



F

Figure 6.7. Scanning Electron Micrographs of verjuice powder after freeze drying for 48h and 72h (magnification at 1000x) (48h freeze-dried powder containing A: MD10%, B: MD15%, C: MD20%; 72h freeze-dried powder containing D: MD10%, E: MD15%, F: MD20%)

Particle size and morphological structure of the verjuice powders may vary depending on the grinding conditions of the cakes after freeze drying. Goula and Adamopoulos (2005) noticed the irregular shaped of the powders had lower bulk density. Similarly, the shape of the freeze-dried verjuice powders having less bulk density (20% MD, 0.32 and 0.47 g/cm^3 for FD 48h and FD 72h) were more irregular in shape when studied under 1000x magnification (Figure 6.7).

Considering all properties of freeze-dried verjuice powders obtained from the different MD concentration and different drying times, the freeze drying for 48 h and 72 h were yielded very stable verjuice powders produced using higher MD concentration (20% MD). However, drying for 72 h was not preferred, because it is a long and very expensive process with a high energy consumption. Therefore, freeze drying of the feed mixture having 20% MD (J+20%MD) for 48 h (FD 48h) was selected as the optimum processing conditions for production of verjuice powders. Powders obtained under these processing conditions used for the following accelerated shelf life study.

6.2.7. Changes in The Quality of Verjuice Powders during Accelerated Storage

Verjuice feed mixtures with 20% MD were freeze dried for 48 h. Then freeze-dried verjuice powders were stored at the accelerated storage conditions (40 °C/90% RH). The changes in the physical properties (moisture content, water activity, bulk density, tapped density, flowability [CI], cohesiveness [HR], solubility, hygroscopicity) and color parameters (L*, a*, b*, C*, h°, BI, ΔE) of powders are given in Figure 6.8 and 6.9. The powders were stored in the PET/AL/PE packages under this storage conditions and all measurements were done by opening a different package for each day. One-way ANOVA analysis was applied to the results for all powder properties. The data were assessed by Tukey comparison test at a 95% confidence interval.

According to Figure 6.8, the moisture content of freeze-dried verjuice powders stayed at the constant level until the 56th day of accelerated storage conditions (3.54-3.70% db), whereas it was slightly increased on the 70th day (4.00% db). The water activity of the powders increased indistinctly from 0.20 to 0.30 during 70 days of storage. Kumar and Mishra (2004) and Koc et al. (2010) indicated that powders have good stability when their moisture content and water activity are lower than 4-5% and 0.2-0.25.

Thus, it was shown that water activity of verjuice powders slightly increased to 0.27 after 14 days, and it stayed at this level (around the critical limits) during 56 days of storage period ($p>0.05$). In contrast, Gac fruit Aril powders reported to be stable with a water activity of 0.38-0.54 (Kha et al., 2010).

The bulk density, tapped density, Carr Index (flowability), Hausner Ratio (cohesiveness), solubility and hygroscopicity of verjuice powders stored at the accelerated conditions were depicted in Figure 6.8. Bulk density and tapped density of verjuice powder were measured as 0.38 g/cm^3 and 0.47 g/cm^3 after freeze drying, then increased to 0.42 g/cm^3 and 0.53 g/cm^3 at the end of 28th day and stayed constant at this value up to 70 days. These results were in line with the study of Erbay et al. (2015). The limits of bulk density and tapped density were reported as 0.3-0.6 g/cm^3 (Erbay et al., 2015). Carr Index (CI) and Hausner Ratio (HR) were calculated in order to determine the flowability and cohesiveness properties of the powders during storage period. According to Figure 6.8, flowability and cohesiveness of the powders were remained constant during 70 days of storage, i.e. Carr Index and Hausner Ratio did not significantly change during storage period. Carr Index and Hausner Ratio of the powders were in the range of 17.13-21.40 and 1.21-1.27, respectively. Consequently, the verjuice powders had a good flowability (CI: 15-20) and approximately low cohesiveness (HR:<1.2) when evaluated according to classification by Jinapong et al. (2008). It was found that good flowability and low cohesiveness behaviors of powders were related with the low moisture content and the high concentration of maltodextrin used as a carrier (Fitzpatrick et al., 2007).

Solubility of verjuice powders were found to be very high (89.59-93.92%) and exhibited stable pattern throughout 70 days under the accelerated storage conditions (Figure 6.8). This is crucial for the quality of the powders, because the higher the solubility resulted in a better soluble powders in water (Vardin & Yasar, 2012). The hygroscopicity of verjuice powders were initially increased from 12.90% to 13.97% at the end of 14th day ($p\leq0.05$), then it showed more stable pattern up to 70 days of storage (13.97-14.5%). The reason of increase hygroscopicity in the verjuice powders could be attributed to the increase in the water activity. Similarly, it was found that the powders containing amorphous sugar were more hygroscopic at high water activity level. Because, these sugars are able to absorb high amount of water making powders more hygroscopic (Jaya & Das, 2004; Oberoi & Sogi, 2015).

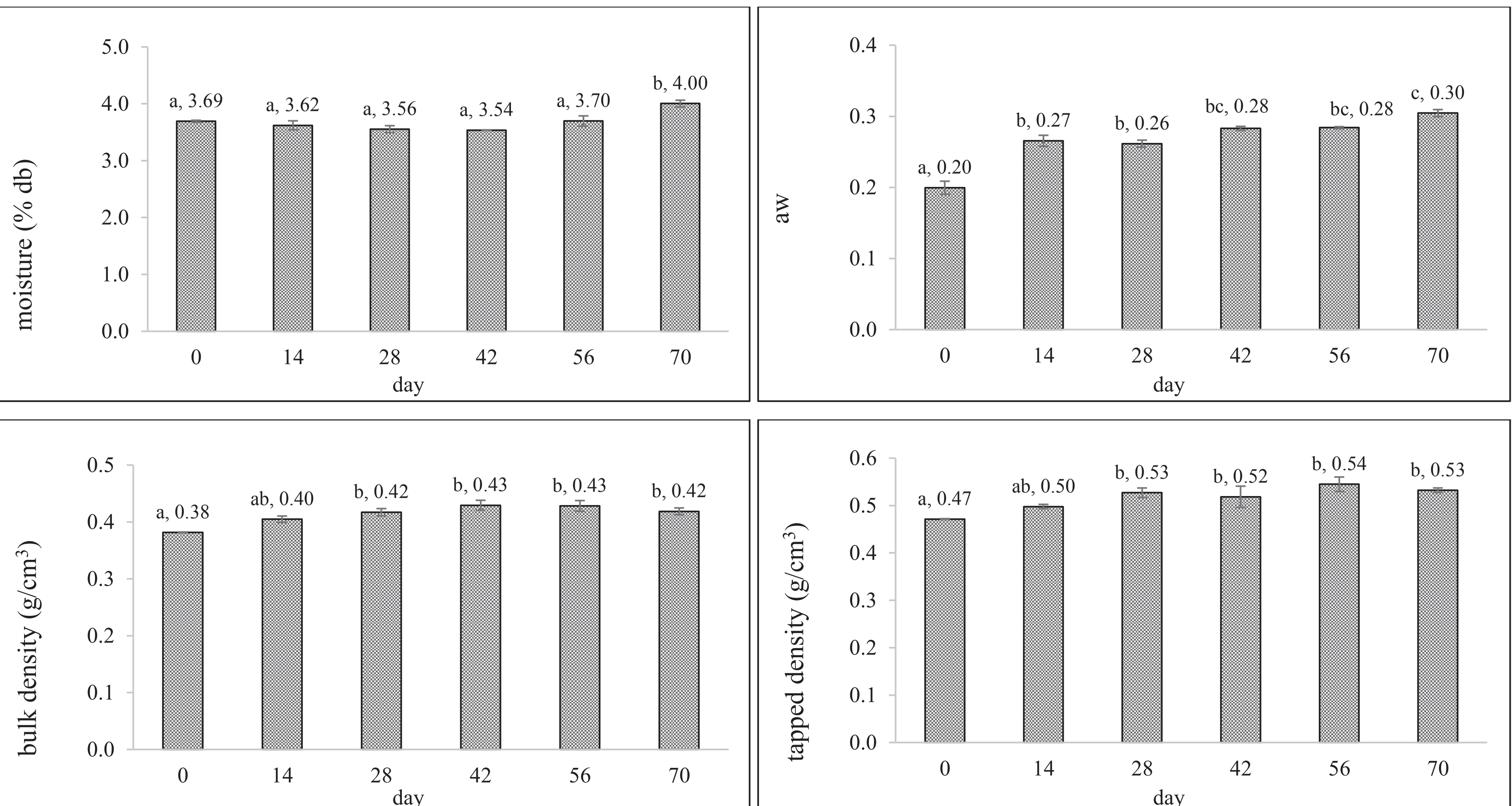
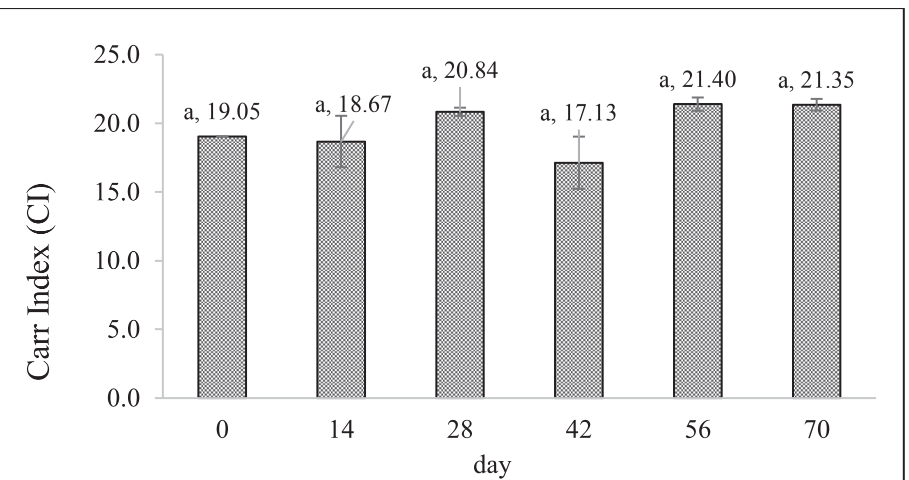
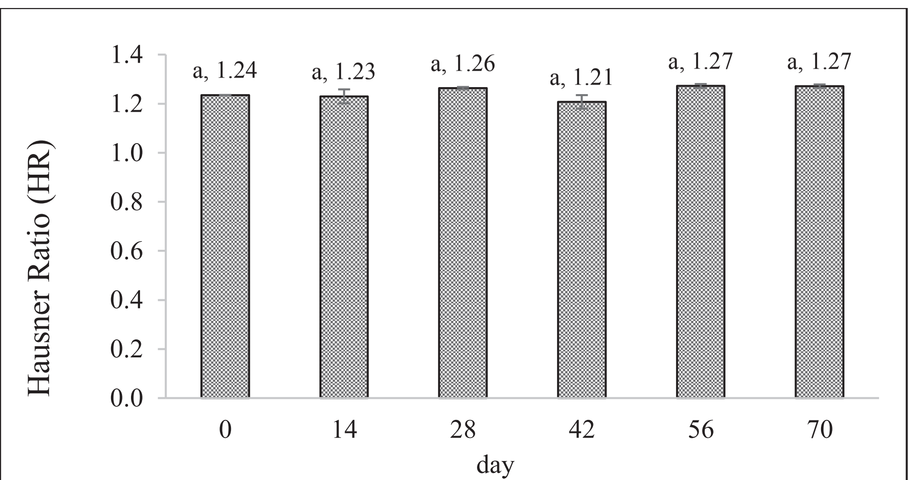
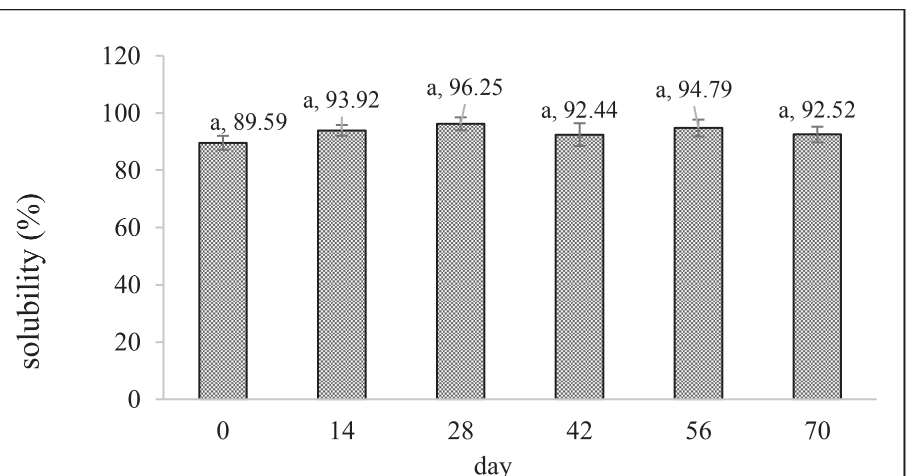


Figure 6.8. Changes in the physical properties of freeze-dried verjuice powder* during the accelerated storage (40 °C/90% RH) of 70 days (*: Powders obtained by drying the J+20%MD feed mixture for 48 h, FD 48h)

(cont. on next page)







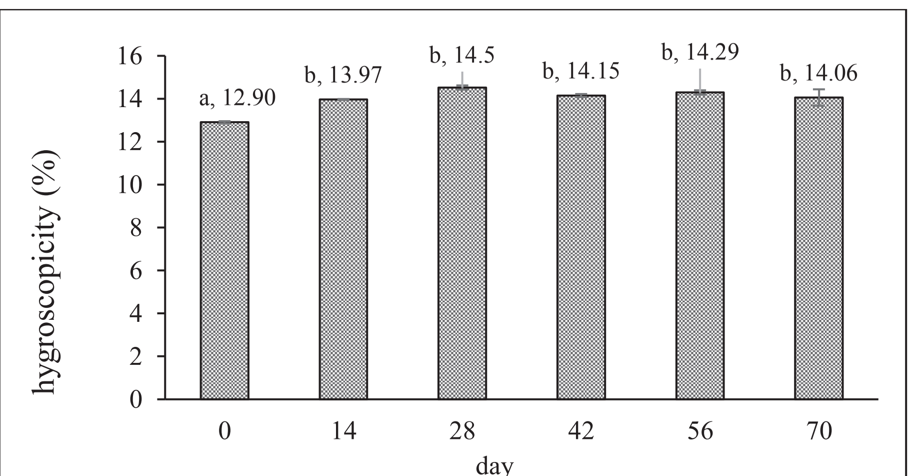


Figure 6.8 (cont.)

Changes in the color parameters, i.e. L*, a*, b*, chroma (C*), hue angle (h°), browning index (BI) and total color difference (ΔE), of freeze-dried verjuice powders were given in Figure 6.9. L* (lightness-darkness) value of verjuice powders did not show an important change up to 42 days. But after 42 days, the powders slightly exhibited darker color. On the other hand, a* (redness-greenness) and b* (yellowness-blueness) values of the samples were significantly increased from 0.51 to 2.91 and from 9.70 to 15.45, respectively, throughout 70 days of storage. These changes in the verjuice powders could be the result of an increase in the water activity value of the samples during the accelerated storage. Similarly, Telis and Martinez-Navarrete (2009) found lower L* and higher a* and b* values in the freeze-dried grapefruit juice powder at the higher water activity levels. They indicated that browning reactions that occur enzymatically or non-enzymatically (Maillard reactions) between phenolic compounds, amino acids, sugars and organic acids could be the reason in powders having high water activity. The value of chroma (C*), which means saturation or color intensity and depends on a* and b* value, were significantly increased from 9.71 to 15.73 until the 70th day (Figure 6.9). This change was similar to the one observed in yellowness (b*) parameter of the powders which was the dominant yellow color of the verjuice. It was also reported that the chroma of the powder was increased with a decrease in L* value (Quek et al., 2007). However, hue angle (h°) of the powder was in the yellow region (87.02°-78.53°) (Pathare et al., 2013) and nearly constant during storage period. Hue angle was estimated from a* and b* values and any change in these parameters during storage would resulted a significant change in hue angle. Since the increasing trend of a* and b* values of verjuice powders during storage was similar, hue angle was not influenced dramatically. Likewise, Topuz et al. (2009) stated that hue angle did not change in freeze-dried paprika powders during storage due to the similar tendency of change observed in a* and b* values. Browning index (BI) of powders was increased from 11.44 to 21.45 at the end of 70 days. BI was estimated from L*, a* and b* values of the samples. In this study, it was concluded that browning occurred during the storage of verjuice powder. Total color difference (ΔE) based on the classification of Cserhalmi et al. (2006) showed that the color change of verjuice powder was slightly noticeable (0-1.5) till the end of 14th day, noticeable (1.5-3) between 14th to 28th day and well visible (3-6) for the rest of storage period (Figure 6.9).

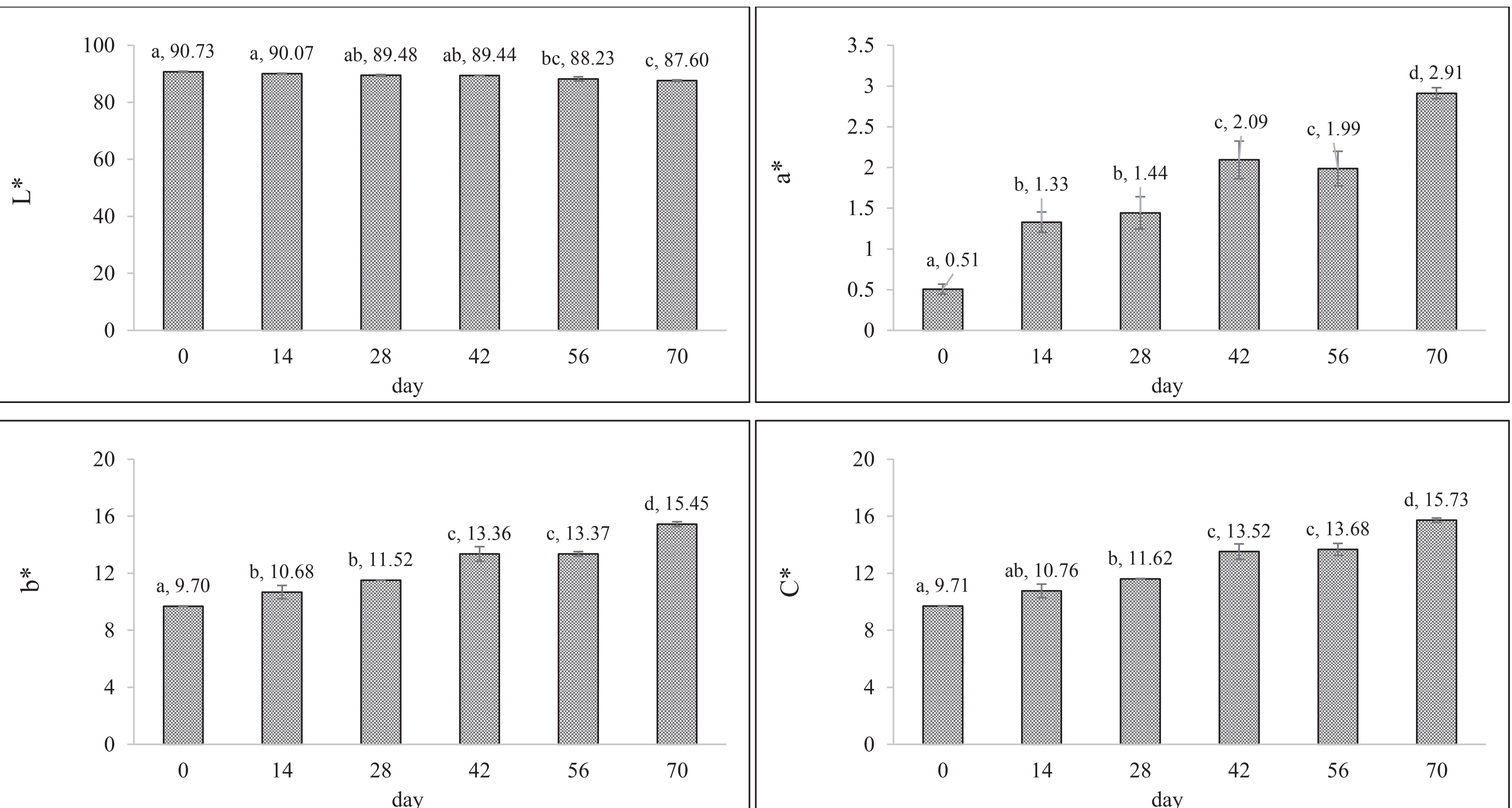
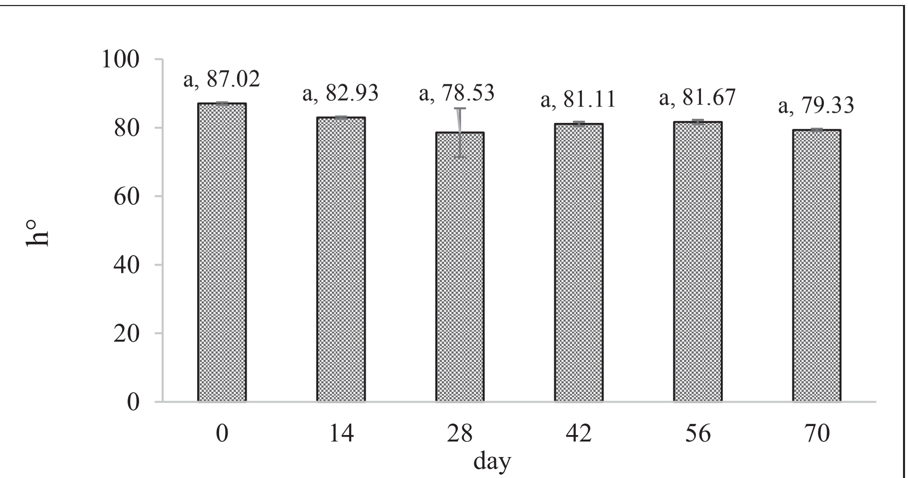
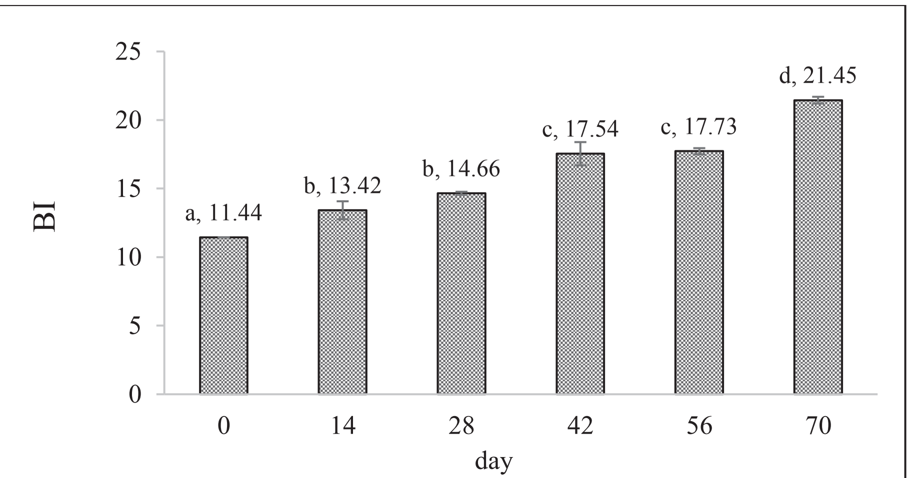


Figure 6.9. Changes in the color properties of freeze-dried verjuice powder* during the accelerated storage (40 °C/90% RH) of 70 days (*: Powders obtained by drying J+20% MD feed mixture for 48 hours, FD 48h)

(cont. on next page)





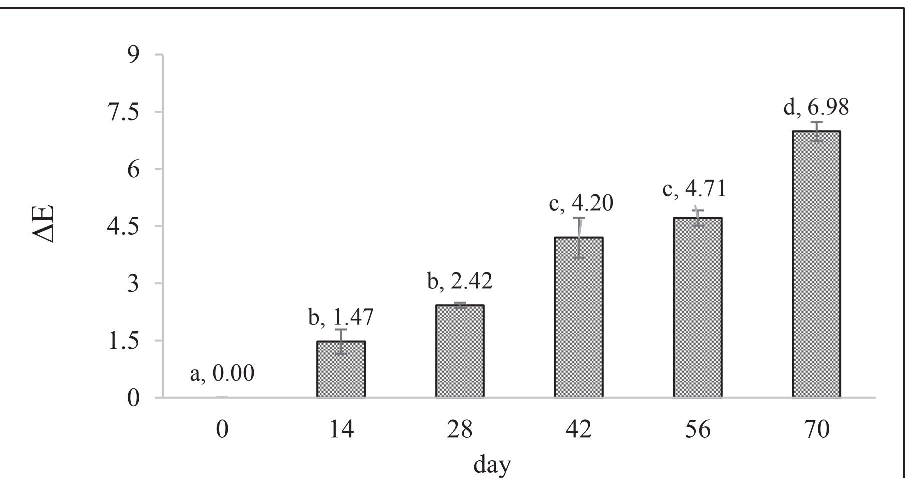


Figure 6.9 (cont.)

6.3. Conclusions

In the first part of this chapter, freshly squeezed verjuice was successfully dried by using freeze drying method. For this purpose, the effect of maltodextrin concentration (10%, 15%, 20% MD) and drying time (FD 36h, 48h, 72h) on the quality properties of freeze-dried verjuice powder were evaluated. The yield of freeze drying method was very high at all MD concentrations and drying times (>96%). Moisture contents and water activity of the powders were significantly decreased by increasing MD concentration and drying time. However, freeze drying of verjuice for 36 h (FD 36h) were resulted in unstable powders with a high moisture content and a_w, at all maltodextrin concentrations. Therefore, powders produced by freeze drying of verjuice feed mixtures with different MD concentrations for 48 h and 72 h (FD 48h and FD 72h) were obtained and analysed for other powder properties. Maltodextrin concentration was not a distinctive factor for evaluating the bulk and tapped densities of powders. However, the bulk and tapped densities of powders produced by FD 72h process were slightly increased at all MD concentrations. All bulk densities of verjuice powders were obtained within the desired limits (0.3-0.6 g/cm^3). Verjuice powder exhibited good flowability and intermediate level of cohesiveness properties. After drying the feed mixture containing 10% MD for 48 hours, the fair flowable and highly cohesive powders were obtained. Solubility and hygroscopicity of powders were changed significantly by increasing MD concentration, but not affected from drying time. The powders became more soluble and less hygroscopic with the higher amount of MD concentration used in the juice. Drying time did not influence the color parameters of the verjuice powders. Higher concentration of the maltodextrin in a feed mixture caused higher L*(lightness), lower a* (redness) and b*(yellowness) value of the powders. Hue angle was not affected by MD concentration and drying time. Lower chroma value were detected in the powders including high amount of maltodextrin in the products. The use of maltodextrin prevented the browning of verjuice powders, i.e. browning index decreased by the increasing of MD concentration. All verjuice powders, particularly the one with 20% MD, was stable at room temperature (25 °C) due to high Tg values. Freeze-dried verjuice powder, showed a skeletal-like structure and was more porous than the other powders obtained by different drying techniques. The particle size of powders became smaller by increasing drying time and maltodextrin concentration. Considering all powder properties, it was concluded that

48 h freeze-dried powder obtained from the feed mixture having 20% MD (J+20%MD, FD 48h) was selected as the optimum conditions to obtain high quality verjuice powder.

In the second part of this chapter, the accelerated shelf life experiments were performed on the verjuice powder obtained at the optimum freeze drying conditions (J+20%MD, FD 48h). The changes in the several properties of the powders within PET/AL/PE packages were monitored during the accelerated storage conditions (40 °C/90% RH) for 70 days. Moisture content was slightly increased during storage but powders were still stable (under the limits of 4-5%) at the end of the 70 days of storage. Water activity of the powders was around the critical limit (0.25) during accelerated storage. Bulk and tapped density of the powders were slightly increased after 28 days, then, they was unchanged until the end of the 70th day. Nevertheless, the changes in bulk and tapped densities were in between the limits (0.3-0.6 g/cm^3). Flowability and cohesiveness of the powders did not change during the accelerated storage. Solubility of verjuice powders were very high (89.59-93.92%) and did not change throughout the storage period. Hygroscopicity of verjuice powders were significantly increased during the accelerated storage conditions. The lower L*, higher a* and b* values in the freeze-dried verjuice powders were detected at the end of the 70 days. Chroma (C*) value was increased, whereas hue angle (h°) of the powder was in the yellow region of 90° during storage. It was concluded that browning occurred during the storage of verjuice powder with increasing the browning index. Total color difference was slightly noticeable (0-1.5) till the end of 14th day, noticeable (1.5-3) between 14th to 28th day and well visible (3-6) for the rest of storage time.

As a conclusion, long shelf life verjuice powder was produced by freeze drying of the feed mixture having 20% MD concentration for 48 h. The powders were highly stable even if the accelerated storage conditions at the higher temperature and relative humidity. Therefore, the powders can be predicted to be much more stable in the storage conditions that have lower temperature and humidity.

CHAPTER 7

PRODUCTION OF CONCENTRATED VERJUICE

Verjuice (unripe grape juice), as an alternative to lemon juice or vinegar, is used in traditional meals, vegetable salads and snacks to give flavour (Hildebrandt & Matchuk, 2002; Oncul & Karabiyikli, 2015). It can be easily spoiled by molds and yeasts due to the traditional production without any technological applications (Hayoglu et al. 2009). As an alternative, verjuice can be consumed in the concentrated form obtained by boiling the juice under household conditions to extend its shelf life. However, traditional boiling method may cause several quality losses in the concentrated products such as degradation of flavour, color and sensorial properties (Cassano et al., 2004; Cevik, Tezcan, Sabanci, & Icier, 2016; Maskan, 2006).

The thermal evaporation is the most common method in which the removal of water is achieved by evaporation under vacuum. Recovered and concentrated volatile compounds are also added back to the final concentrated juice product (Barbe et al., 1998; Yaakob, 2012). It is a stable and economical method results in less storage, smaller size of packaging and lower transportation costs (Bozkir & Baysal, 2017). However, thermal degradation of the nutrients and cooked flavour in the concentrated juice product are the disadvantages of the evaporation process (Cassano & Drioli, 2007). In order to avoid the effects of overheating, vacuum is applied by reducing the pressure during the concentration process. Boiling point of the liquid decreases and vapor transfer occurs more rapidly at lower temperature under vacuum. This method also maintains the nutritional, color, flavor of the product (Bozkir & Baysal, 2017). There are many published dealing with the vacuum concentration of juices. Physical, phytochemical, nutritional, sensorial and rheological properties of concentrated juice products are well documented (Assawarachan & Noomhorm, 2010; Boranbayeva, Karadeniz, & Yilmaz, 2014; Bozkir & Baysal, 2017; Burdurlu & Karadeniz, 2003; Cevik et al., 2016; Dincer et al., 2016; Fazaeli et al., 2013b; Gollucke, De Souza, and De Queiroz Tavares, 2008; Kaya & Sozer, 2005; Maskan, 2006).

In this chapter, concentrated verjuice is produced by two different evaporation processes including vacuum evaporation and boiling at atmospheric pressure. In the first

part of this chapter, freshly squeezed verjuice was concentrated up to 65 °Brix at three different boiling temperature/vacuum pressure combinations, i.e. 45 °C/913 mbar, 55 °C/855 mbar and 65 °C/763 mbar. Evaporation at the atmospheric pressure (open-pan boiling at 100 °C) was used to produce concentrated verjuice in order to mimic the evaporation process of verjuice in household conditions. Several quality characteristics (pH, TSS, titratable acidity, color, water activity, moisture content, density, viscosity, total phenolic content, non-enzymatic browning index [NEBI] and hydroxymethylfurfural [HMF]) of evaporated samples were analysed and compared to determine the optimum conditions for the evaporation process. Finally, some quality parameters of concentrated verjuice obtained under the optimum evaporation conditions, were evaluated during the storage period at room temperature (25 °C).

7.1. Materials and Methods

7.1.1. Concentration Process of Verjuice

Freshly squeezed verjuice was concentrated using vacuum evaporation and open-pan evaporation methods. A rotary vacuum evaporator (A Laborota 4001-Efficient, Heildoph Instruments, GmbH & Co., Germany) equipped with a vacuum pump (Rotavac, Heildoph Instruments, GmbH & Co., Germany) and a cooler system (Rotacool, Heildoph Instruments, GmbH & Co., Germany) located in the Biotechnology and Bioengineering Research and Application Centre in İzmir Institute of Technology was used for the concentration of verjuice. Evaporation at atmospheric pressure (open-pan boiling) was also employed for the production of verjuice concentrate. The main steps of production of concentrated verjuice is given in Figure 7.1.

Freshly squeezed verjuice (4.29±0.07°Brix) was prepared based on the procedure explained in Chapter 3 (section of 3.1.2). Five hundred millilitres (500 mL) of verjuice placed in a glass jar (1 L) was concentrated at the rotary evaporator under different boiling temperature/vacuum pressure conditions, i.e., 45 °C/913 mbar, 55 °C/855 mbar and 65 °C/763 mbar, respectively. Rotary speed was 100 rpm for all processes. Additionally, the same amount of verjuice (500 mL) in 1 L of glass jar was evaporated by using a heater at the atmospheric pressure conditions (at 100 °C).

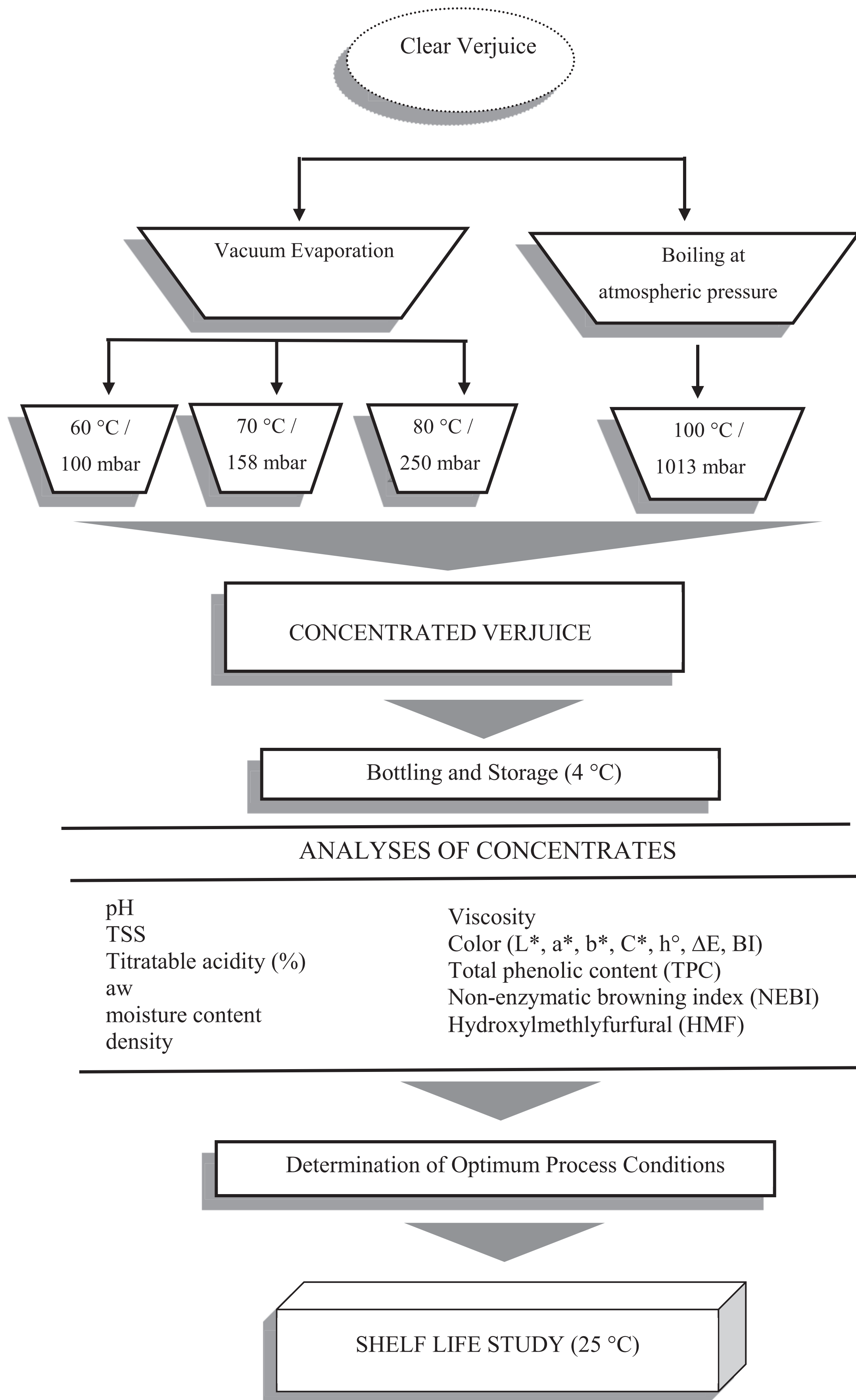


Figure 7.1. The schematic diagram of production of the concentrated verjuice

Final total soluble solid (TSS) content of concentrated verjuice samples, after all evaporation processes, was fixed to 65 g/100 g (65°Brix), by taking literature and commercial specifications of concentrated product into account (Cassano & Drioli, 2007; Dincer et al., 2016; Goldenmeyve, 2012; Ibarz et al., 2009; Vaillant et al., 2001). After the evaporation processes, concentrated verjuice samples were stored in the air-tied falcon tubes in the refrigerator prior to analyses. All the process conditions for the vacuum evaporation of verjuice are given in Table 7.1. All experiments were repeated three times.

The evaporation rate of verjuice was calculated after each evaporation processes, i.e. vacuum and conventional evaporation methods. Several quality parameters of concentrated verjuice were measured after all evaporation treatments in Figure 7.1.

Table 7.1. Processing conditions of verjuice evaporation

Process	Experiment	$T_{operational}$ (°C)	$P_{absolute}$ (mbar)	$T_{boiling}$ (°C)	P_{vacuum} (mbar)	ΔT (°C)
Vacuum Evaporation (VE)	1	60	100	45	913	15
	2	70	158	55	855	15
	3	80	250	65	763	15
Open-pan Evaporation (OPE)	1	100	1013	100	-	-

7.1.2. Evaporation Rate

Evaporation rate required to achieve the target °Brix, which was defined as the amount of water loss from the juice per unit time, was calculated according to Bozkir and Baysal (2017) (Equation 7.1). The amount of evaporated water (g) in verjuice was plotted against time, where the slope of the curve was the evaporation rate, expressed as "g H_2O/min". Additionally, the correlation between the total soluble solid content of verjuice and concentration time was interpreted with an exponential model. The evaporation rate constant (k), was also calculated from the first order model given in the Equation 7.2 (Assawarachan & Noomhorm, 2008).

$$Evaporation\ rate = \frac{\Delta x}{\Delta t} \tag{7.1}$$

$$B_t = B_0 \pm \exp(kt) \qquad \text{or} \qquad k = \frac{\ln(\frac{B_t}{B_0})}{t} \tag{7.2}$$

where k is the evaporation rate (min^{-1}), t is the concentration time (min), Δx is the loss of water in verjuice (g), B_0 is initial soluble solid content of verjuice before evaporation (°Brix), B_t is soluble solid content of verjuice after t time of concentration at a given temperature (°Brix).

7.1.3. Physicochemical Analyses of the Concentrated Verjuice

Physicochemical properties of concentrated verjuice, i.e. pH, total soluble solid content (°Brix), titratable acidity (with a 1 mL of concentrated sample), and color (L*, a*, b*, hue°, chroma*, BI, ΔE) (with a 30 mL of reconstituted sample) were performed as described in Chapter 4 (section 4.1.5) with a slight modifications in the sample amounts. Moisture content and water activity of the concentrates were also determined according to the procedures given in Chapter 6 (section 6.1.2).

7.1.3.1. Density

A portable type density meter (Kyoto Electronics DA, Japan) was used to measure the density of the samples at room temperature (20°C). Approximately 10 mL of concentrated verjuice was filled to the tube of the device and the density value was directly read as "g/cm^3".

7.1.3.2. Viscosity

Viscosity is a measure of a fluid's resistance to flow and it is defined as "the ratio of shearing stress applying to the rate of shear produced". (Barker, 2008). This ratio is constant for Newtonian fluids. The viscosity measurements of concentrated verjuice samples were performed by using a rotational viscometer (Haake VT 550 Viscotester, Thermo Inc., Germany), equipped with a coaxial cylinder sensor SV-DIN and a thermocontroller water bath (DC30, Haake, Thermo Inc., Germany). Flow curves were

obtained by an upward/downward shear rate ramp test to determine time dependency of the samples. For this purpose, 10 mL of concentrated juice sample was placed into the SV-DIN rotor of the viscometer and shear rate was increased from 0 to 600 1/s, and then immediately reduced from 600 to 0 1/s. Temperature was set to 25 °C by using the equipment's thermocontroller water bath. It was observed that shear stress of the samples linearly increased by increasing the shear rate, and decreased by reducing the shear rate. Therefore, Newtonian model given in Equation 7.3 was applied to determine the viscosity. Shear stress (σ, Pa) data were plotted against the shear rate ($\acute{Y}$, 1/s). The viscosity values of the samples (η, Pa.s) were calculated from the slope of the flow curve and expressed as "Pa.s".

$$\sigma = \eta * \acute{Y} \quad (7.3)$$

7.1.3.3. Total Phenolic Content

Total phenolic compounds (TPC) of the concentrated verjuice were determined by Folin-Ciocalteu method (Singleton & Rossi, 1965; Tezcan, Gultekin-Ozguven, Diken, Ozcelik, & Erim, 2009) with some modifications. Concentrated verjuice sample (65°Brix) was firstly reconstituted with addition of distilled water at a ratio of 1:20 up to total soluble solid content of 4.3°Brix. Then, reconstituted juice was diluted at a ratio of 1:30 with methanol:water solution (3:2); and it was centrifuged at 5000 rpm for 5 min (4 °C) for the extraction of polyphenols and clarification of the juice. 300 µL of supernatant was put into the glass tubes. 1.5 mL of Folin-Ciocalteu reagent diluted with distilled water (1:10) and 1.2 mL of sodium carbonate solution prepared with distilled water (75 g/L) were added to the tubes within a very short time (approximately 5 min). The mixtures were shaken by a vortex mixer and stored for 90 min under dark conditions at room temperature (20 °C). Blank was also prepared in the same procedure by using 300 µL of methanol:water solution (3:2) instead of the sample. Absorbance of the samples were recorded at a wavelength of 760 nm after zeroing the spectrophotometer (Carry 100 UV-Visible Spectrophotometer, Varian Inc., CA, USA) with the methanol:water solution (3:2). Standard curve prepared with different concentrations of gallic acid (up to 150 ppm) was generated with the linear equation of y=0.0117x+0.0161 where y is the absorbance (cm^{-1}) and x is the gallic acid concentration (ppm) with R^2 value of 0.9992. The phenolic

contents of the concentrated verjuice samples were calculated as gallic acid equivalent (GAE) (g GAE/L).

7.1.3.4. Non enzymatic browning index (NEBI)

Non-enzymatic browning reactions occurred chemically or physically can be used as an index or parameter to evaluate the degree of the quality changes in the products (Cohen, Birk, Mannheim, & Saguy, 1998). Non-enzymatic browning index (NEBI) of the concentrated verjuice samples were determined based on the method used by Keeney and Bassette (1959) and Cohen et al. (1998). In this method, concentrated verjuice was firstly reconstituted with distilled water to reach the total soluble solid content of freshly squeezed verjuice (4.3°Brix). 5 mL of reconstituted juice was mixed with a 5 mL of ethanol (95% v/v). The mixture was centrifuged at 7800 x g for 10 min (4 °C). The supernatant of the juice mixture was divided into two parts for NEBI and HMF analyses. Absorbance value of the first part was directly read against water at 420 nm wavelength by using the spectrophotometer (Carry 100 UV-Visible Spectrophotometer, Varian Inc., CA, USA). The observed value is expressed as the non-enzymatic index (NEBI).

7.1.3.5. Hydroxymethylfurfural (HMF)

The amount of Hydroxymethylfurfural (HMF) in the concentrated verjuice samples was determined according to Keeney and Bassette (1959) and Cohen et al. (1998). The second part of the supernatant obtained in the NEBI analysis (section 7.1.2) was used for the HMF analysis. 2 mL of the supernatant, 2 mL of trichloroacetic acid (TCA, Merck, Darmstadt, Germany) in the concentration of 12% (w/v) and 2 mL of thiobarbutiric acid (TBA, Sigma-Aldrich Corp., St. Louis, MO) in the concentration of 0.025 mol/L were placed into the glass tubes and mixed thoroughly using a vortex. The tubes were kept in the water bath fixed at 40 °C for 50 min in the dark. After the waiting period, the tubes were immediately cooled within cool water; and the absorbance of the tubes were read against water at 443 nm wavelength in the spectrophotometer (Carry 100 UV-Visible Spectrophotometer, Varian Inc., CA, USA). Standard HMF curve was obtained by measuring the absorbance of the HMF solutions prepared with different concentrations in the range of 0-28 ppm. The amount of HMF was expressed as “mg/kg”.

7.1.4. Shelf Life Study

The concentrated verjuice sample obtained at the optimum conditions of the evaporation process was used for the shelf life study. For this purpose, freshly squeezed verjuice (500 mL) was evaporated by using the rotary vacuum evaporator in the selected conditions (45 °C/913 mbar), until the final total soluble solid content of 65°Brix. The concentrated samples were transferred into the dark-colored glass tubes and placed inside the incubator (TK 120, Nüve, Ankara, Turkey) at room temperature (25 °C) for 12 weeks. The quality parameters of the concentrated verjuice such as color (L*, a*, b*, hue°, chroma*, BI), total phenolic content (TPC), non-enzymatic browning index [NEBI] and hydroxymethylfurfural [HMF]) were monitored periodically with 2-weeks intervals (0, 2, 4, 6, 8, 10, 12th week) at 25 °C. Additionally, concentrated samples were also stored at refrigerated conditions (4 °C). Physical and chemical properties of samples stored at 4 °C and 25 °C were compared at the end of shelf life (12th week).

7.1.4.1. Kinetic modelling of color parameters during storage

Change in the quality of a food product during processing and storage, the kinetic modeling is applied to some quality parameters of the food and the reaction rate of this kinetics is evaluated (Van Boekel, 1996). Degradation of color in concentrated fruit juices is mostly explained by zero-order and first-order reaction kinetics in the literature. Formation of colored polymeric compounds (Maillard reactions etc.) explained by zero-order reaction kinetics and degradation of colored pigments to discolored form (destruction of natural color compounds etc.) was defined by first-order reaction kinetics (Maskan, 2006; Van Boekel, 2008). These kinetics were used to detect the color change of concentrated verjuice during 12 weeks of refrigerated (4 °C) and room (25 °C) storage conditions, respectively. The zero-order and first-order kinetic equations are expressed by linear and exponential models in Equation 7.4 and 7.5, respectively.

$$C_t = C_0 \pm k_0 t \tag{7.4}$$

$$C_t = C_0 \exp(\pm k_1 t) \tag{7.5}$$

where (+) and (−) referred formation and degradation of any color parameters. C_0 and C_t is the color parameter of verjuice at initial and storage time t (week), respectively. k_0 (min^{-1}) and k_1 (min^{-1}) is the zero-order and first-order kinetic constants.

7.1.5. Statistical Analysis

The concentrated verjuice samples that were produced by using vacuum and open-pan evaporation methods were statistically analysed by using One-way analysis of variance (ANOVA). ANOVA were carried out for evaporation processes to determine how significantly the independent variables affect the dependent variables. Independent variables were vacuum evaporation conditions that were 45 °C/913 mbar, 55 °C/855 mbar and 65 °C/763 mbar and atmospheric condition that was 100 °C. Dependent variables were quality parameters of the concentrated verjuice samples (pH, total soluble solid content, titratable acidity, color, total phenolic content, NEBI and HMF). All results were given with their means and standard deviations. Least significant difference (LSD) test was applied and the means of data were compared by Tukey's pairwise comparison test. Minitab 16 software program (Minitab Inc., State College, PA, USA) was employed for one-way ANOVA and least significant difference test of the results. All processing experiments were repeated three times and the measurements in shelf life study was repeated two times.

7.2. Results and Discussion

7.2.1. Evaporation Rate

The verjuice was concentrated up to 65 °Brix by vacuum evaporation method processed at different boiling temperature and vacuum pressure conditions, along with the open-pan method at atmospheric pressure. The changes in the total soluble content of verjuice (°Brix) during evaporation are shown in Figure 7.2. The average concentration time, up to 65 °Brix, were recorded as 123 min, 108 min and 101 min at 45 °C/913 mbar, 55 °C/855 mbar and 65 °C/763 mbar, respectively. Likewise, verjuice was boiled at 100 °C (atmospheric pressure) for 133 min to reach the same soluble solid content (65 °Brix).

Similarly, Maskan (2006) found that the boiling of pomegranate juice at atmospheric pressure was longer than the concentration process using the rotary evaporator at 40 °C.

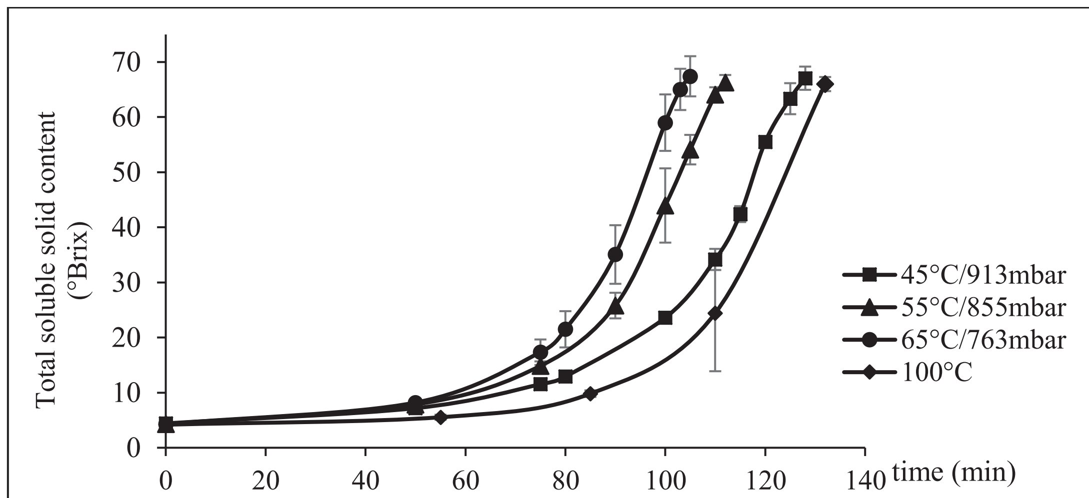


Figure 7.2. Change in verjuice concentrate (°Brix) produced by various concentration processes

The evaporation rates of verjuice were calculated as; 3.80 g H_2O/min at 45 °C/913 mbar, 4.34 g H_2O/min at 55 °C/855 mbar, 4.64 g H_20/min at 65 °C/763 mbar and 3.53 g H_2O/min at 100 °C ($R^2>0.97$) (Table 7.2). The amount of water evaporated per unit time is estimated to be a low at the low temperature. However, the evaporation rate at 45 °C/913 mbar vacuum condition was similar to the evaporation rate at atmospheric pressure ($p>0.05$) (Table 7.2). Vacuum application during evaporation process shortened the concentration time by allowing rapid removal of the steam from the juice. Furthermore, the evaporation rate at 55 °C/855 mbar and 65 °C/763 mbar were found to be significantly higher than the vacuum operation at 45 °C/913 mbar. The higher processing temperatures were yielded higher evaporation rates. The longest evaporation process was achieved at the atmospheric pressure and the shortest one was achieved at 65 °C and 763 mbar vacuum pressure. Therefore, it can be concluded that the removal of water from verjuice, depended significantly on the process temperature and vacuum pressure. Evaporation rate of verjuice at 65 °C/763 mbar (4.22 gH_20/min) reported in this study was in agreement with the evaporation rate of apple juice processed at 82 °C/500 mbar (4.33 gH_2O/min) stated by Bozkir and Baysal (2017). The reason of slightly higher evaporation rate calculated in their study could be the higher process temperature employed. Additionally, Al-Mutairi and Al-Jasser (2012) found that rotary vacuum evaporation was faster than boiling at 100 °C in the concentration of date juice. They

declared that this difference was due to being the mixing effect of rotary evaporator and also reduction in the boiling point of water by the effect of vacuum pressure.

Table 7.2. Evaporation rate and evaporation rate constant of verjuice during evaporation processes at different conditions

$T_{boiling}$ (°C)	P_{vacuum} (mbar)	Evaporation rate (ΔX/Δt)			Evaporation rate constant (B_t=B_0 + exp [kt])			Evaporation time (min)
		Rate (g H_2O/min)		R^2	k (min^{-1})		R^2	
45	913	3.80	±0.05a	0.99	0.036	±0.002ab	0.99	123 ±2
55	855	4.34	±0.05b	0.99	0.042	±0.002bc	0.99	108 ±1
65	763	4.64	±0.10b	0.99	0.048	±0.001c	1.00	101 ±2
100	-	3.53	±0.31a	0.96	0.031	±0.005a	0.97	133 ±11

(Different small letters in the same line indicate differences between samples based on One-way ANOVA analyses compared by Tukey test ($p \leq 0.05$). ΔX: the loss of water in verjuice (g H_20), Bo: initial TSS content of verjuice (°Brix), Bt: TSS content of verjuice (°Brix) after t time)

The evaporation rate constants (k) of all process conditions were also reported in Figure 7.3 and Table 7.2. The rate constant of verjuice at 45 °C/913 mbar (k: 0.036 min^{-1}) and 65 °C/763 mbar (k: 0.048 min^{-1}) were statistically different from each other ($p \leq 0.05$), whereas the rate constant calculated at 55 °C/855 mbar (k: 0.042 min^{-1}) was similar to each of these treatments ($p > 0.05$). Thus, the increase of boiling temperature to 65 °C markedly accelerated the evaporation of verjuice and reduced the concentration time. Unlike vacuum evaporation processes, the open-pan method decelerated the concentration of verjuice with the lowest evaporation rate constant (0.031 min^{-1}). Several literature explained the relation between evaporation rate constant and process time by using different evaporation method employed under various conditions (Assawarachan & Noomhorm, 2008; Bozkir & Baysal, 2017; Fazaeli et al., 2013b; Maskan, 2006). Bozkır and Baysal (2017) reported that time could be reduced by using higher vacuum pressure during concentration, as a result of water's boiling point being reduced and evaporation occurring in a faster way. Correspondingly, Fazaeli et al. (2013b) observed higher evaporation rate constant (0.010 min^{-1}) for black mulberry juice evaporated under vacuum pressure (P_{vacuum}: 940 mbar) than the ones operated at atmospheric pressure (0.006min^{-1}). Assawarachan & Noomhorm (2008) found the evaporation rate constant of pineapple juice as 0.040 min^{-1} at 55 °C/80 kPa (P_{vacuum}: 213 mbar). The declared evaporation rate was slightly lower than the one obtained at 55 °C under higher vacuum condition (P_{vacuum}: 855 mbar) used in this study.

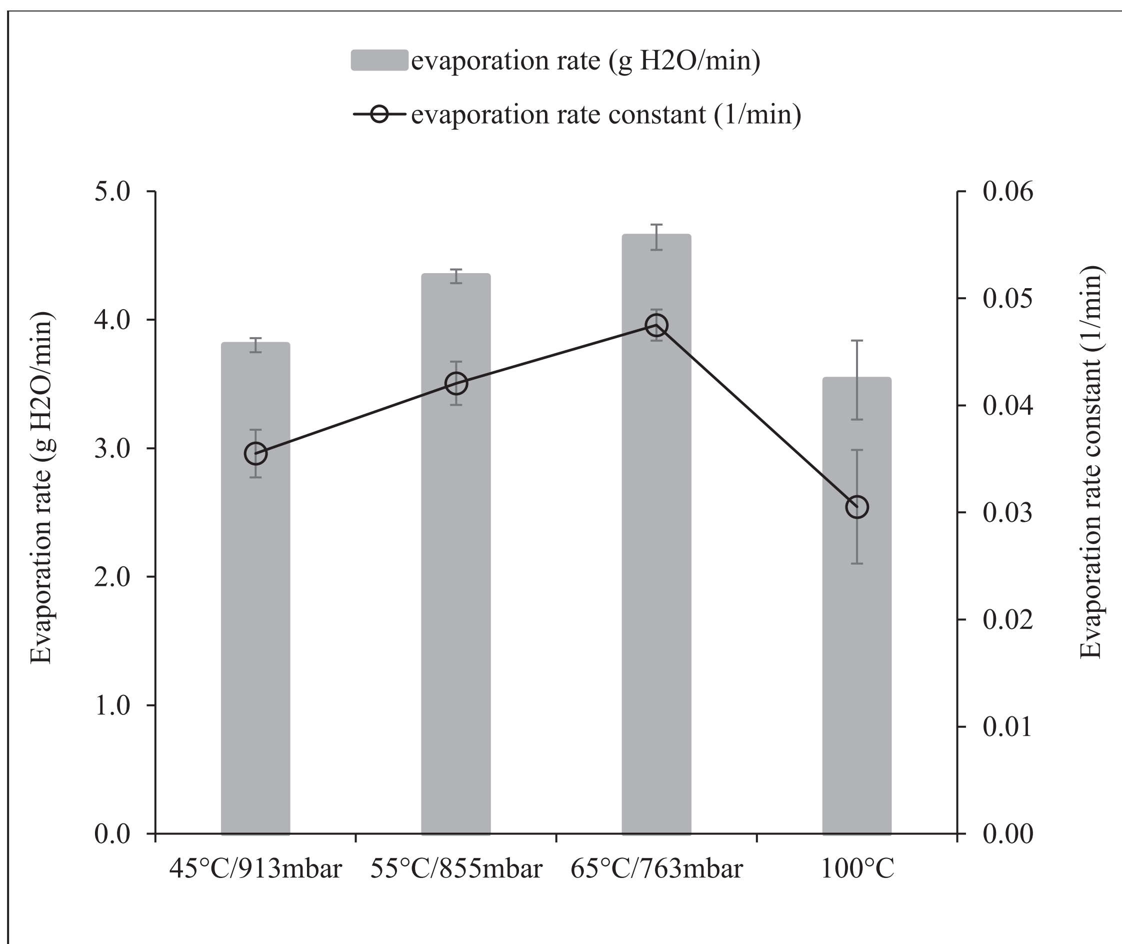


Figure 7.3. Change in evaporation rate (grey bar) and evaporation rate constant (black line) of verjuice concentrate produced by various concentration processes

7.2.2. Physicochemical Properties of Concentrated Verjuice

Several physical quality properties of the concentrated verjuice (pH, TSS, titrable acidity, density, water activity, moisture content, viscosity) at 45 °C/913 mbar, 55 °C/855 mbar, 65 °C/763 mbar as well as evaporation at atmospheric pressure were listed in Table 7.3. According to Table 7.3, all physical parameters of concentrated verjuice were not significantly different from each other ($p>0.05$).

Total soluble solid content of verjuice was initially constant (4.29±0.07 °Brix), and it was raised to approximately 65 °Brix by different evaporation processes. Therefore, the moisture contents (32.59-34.78%) and water activities (0.72-0.74) of verjuice were found to be similar in the samples having the same solid content. pH and acidity of concentrated samples were actually not affected by temperature or pressure used in this study, but the amount of acidity per liter of verjuice (g/100mL) was slightly increased by

various concentration processes. This increament in acidity content was not statistically significant. In constrast, Gurak, Cabral, Rocha-Leao, Matta, and Freitas (2010) and Vaillant et al. (2001) reported the increase of acidity in concentrated grape juice comparing to untreated juice.

Table 7.3. Physicochemical properties of concentrated verjuice produced by various evaporation processes

	45 °C/ 914 mbar		**55 °C/ 855 mbar**		**65 °C/ 763 mbar**		**100 °C (OPE)**	
pH	1.35	±0.02a	1.32	±0.01a	1.32	±0.00a	1.32	±0.04a
TSS (°Brix)	64.88	±0.18a	65.33	±0.67a	65.17	±0.55a	65.47	±0.85a
T.A (%)	58.73	±3.80a	59.94	±1.70a	61.15	±0.90a	58.79	±1.43a
Density (g/cm^3)	1.35	±0.01a	1.35	±0.01a	1.35	±0.01a	1.36	±0.02a
a_w	0.74	±0.01a	0.74	±0.01a	0.73	±0.01a	0.72	±0.01a
Moisture (%)	33.67	±0.41a	33.16	±1.10a	32.59	±1.38a	34.78	±1.27a
Viscosity (η, Pa.s)	0.12	±0.02a	0.13	±0.01a	0.13	±0.01a	0.14	±0.02a

Different small letters in the same line indicate differences between samples based on One-way ANOVA analyses compared by Tukey test ($p \leq 0.05$).

Rheological properties of the concentrated juice products is important from the quality, operating parameters and consumer acceptance point of view (Diamante & Umemoto, 2015; Kaya & Belibagli, 2002; Rao, 1999). Since the change of temperature affects the flow characteristics of fruit juices, rheological behavior of the thermally treated juice must be analysed to evaluate the evaporation process (Crandall, Chen, & Carter, 1982). With regard to the rheological properties, it was found that density and viscosity values of concentrated verjuice samples were not significantly from each other ($p>0.05$) (Table 7.3). The reason of this was due to having the same total soluble solid contents (65 °Brix) of each samples. The concentrated verjuice samples exhibited Newtonian behavior at room temperature (25 °C) since the linear relationship was found between shear stress and shear rate data with a very high R^2 value. Viscosity values were in the range of 0.12-0.14 Pa.s (Table 7.3). Flow curves of the concentrated samples were depicted in Appendix D (Figure D.1). Ibarz et al. (2009) reported that the flow behavior of fruit juices depended on temperature and total soluble solid content. They observed Newtonian behavior in the

concentrated clarified orange juice having 65.8 °Brix and a similar viscosity value (0.10 Pa.s) at 25 °C. The rheological behavior of concentrated pomegranate juice (70 °Brix) and concentrated grape juice (66.8 °Brix) at different temperatures were also reported as the Newtonian (Kaya & Belibagli, 2002; Kaya & Sozer, 2005). Cevik et al. (2016) found that the viscosity of unripe grape concentrates (25 °Brix) was $2.7*10^{-3}$ Pa.s at 25 °C. Their result was much lower than the viscosity value measured in this study due to the different soluble solid content of the concentrated samples. Cevik et al. (2016) reported that flow behavior of the unripe grape juice depended on the total soluble solid content and the amount of insoluble suspended solids. They observed non-Newtonian behavior in the concentrated samples at the lower soluble solid contents (<20 °Brix). However, the flow characteristics of the samples at higher concentration (25 °Brix) was explained by the Newtonian model. They also indicated that tartrate crystals in the concentrates might cause non-Newtonian behavior in the concentrated samples. However, the concentrates in this study were detartarized and exhibited Newtonian flow behavior.

7.2.3. Color Analyses of Concentrated Verjuice

Several color parameters (L*, a*, b*, h°, C*, ΔE, BI, NEBI) of concentrated verjuice samples were compared with untreated verjuice in order to evaluate the effects of different evaporation conditions on the sample color (Table 7.4). Generally, concentrated verjuice obtained by open-pan method at 100 °C was dramatically different from the vacuum evaporated samples in terms of its redness (a*), non-enzymatic browning index (NEBI) and total color difference (ΔE) (Figure 7.4). The open-pan concentrated verjuice showed lower lightness (L*) and hue angle (h°) as well as higher chroma (C*) and browning index (BI). They were significantly different from the ones obtained at lower temperatures under vacuum conditions ($p \leq 0.05$) (Table 7.4). Therefore, it was concluded that evaporation at high temperature caused browning of the juice and resulted in a visible red color in the concentrated samples. Since color of the open-pan evaporated sample was very dark, the color properties of the vacuum evaporated verjuice samples were compared with each other after this point. Considering the color of the vacuum evaporated samples, all of them possessed different color. However, the concentrated verjuice at 45 °C/914 mbar exhibited the closest color characteristics to the untreated juice (Figure 7.4).

Table 7.4. Color parameters of concentrated verjuice* produced by different evaporation processes

	Untreated Juice	45 °C/ 914 mbar	55 °C/ 855 mbar	65 °C/ 763 mbar	100 °C (OPE)
L*	29.25 ±0.06a	26.81 ±0.17b	26.59 ±0.16bc	26.03 ±0.04c	20.31 ±0.46d
a*	0.14 ±0.03a	0.88 ±0.05ab	1.06 ±0.18ab	1.74 ±0.12b	7.36 ±0.44c
b*	2.84 ±0.38a	7.18 ±0.45b	7.38 ±0.50b	7.91 ±0.08b	4.26 ±0.25c
C*	2.84 ±0.38a	7.24 ±0.45b	7.45 ±0.52bc	8.10 ±0.07bc	8.51 ±0.42c
h°	87.12 ±0.37a	83.03 ±0.02b	81.88 ±0.90b	77.61 ±0.93c	29.90 ±2.14d
BI	10.21 ±1.47a	32.58 ±2.28b	34.43 ±3.25bc	39.97 ±0.24c	48.25 ±1.92d
ΔE	0.00 ±0.00a	3.36 ±0.47b	3.58 ±0.52b	4.30 ±0.04c	9.44 ±0.48d
NEBI	0.08 ±0.00a	0.14 ±0.01b	0.15 ±0.01b	0.18 ±0.00c	1.19 ±0.18d

(Different small letters in the same line indicate the differences between samples based on One-way ANOVA analyses compared with Tukey test ($p \leq 0.05$). *: reconstituted verjuice samples with a dilution factor of 14.13)

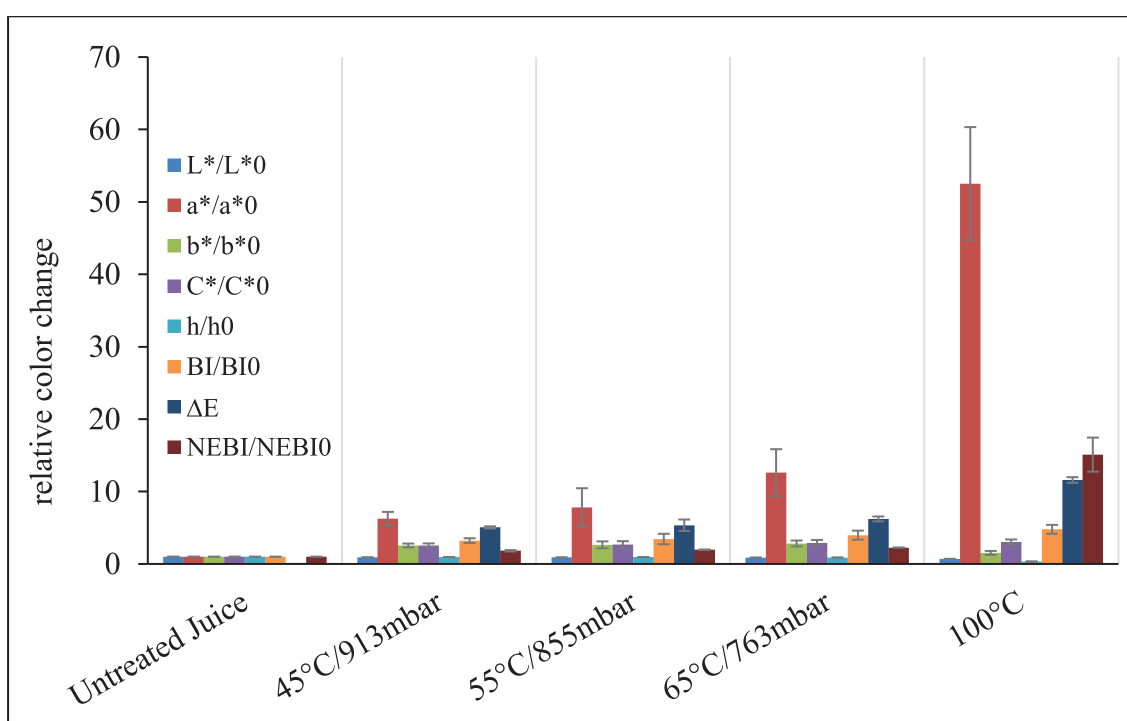


Figure 7.4. The change in color parameters of concentrated verjuice* produced by different evaporation processes (*: reconstituted verjuice samples with a dilution factor of 14.13)

L* and a* values of concentrated verjuice at 45°C/914 mbar and 65°C/763 mbar were significantly different from each other ($p \leq 0.05$), whereas b* values were similar

(Table 7.4). In other words, the concentrated samples became darker (L*changed from 26.81 to 26.03) and redder (a* changed from 0.88 to 1.74) by increasing the processing temperature from 45 °C to 65 °C. The color properties of the concentrated verjuice at 55 °C/855 mbar were not different from the samples processed at 45 °C/914 mbar and 65 °C/763 mbar (Table 7.4). According to the classification of hue angle given by Pathare et al. (2013), the color of concentrated verjuice at 65 °C (h°: 77.61) was closer to red compared to the concentrates obtained at 45 °C (h°: 83.03) and 55 °C (h°: 81.88). This was because the sugars in the verjuice composition caramelized during evaporation at high temperatures and the color was found to be close to red (Assawarachan & Noomhorm, 2010). Saturation of the vacuum concentrated samples remained stable due to the similar chroma values.

According to Rattanathanalerk, Chiewchan, and Srichumpoung (2005), enzymes causing browning reactions were mostly inactivated at the processing temperature higher than 50 °C. Therefore, non-enzymatic browning reactions and pigment destruction occurred in the evaporation conditions in this study, especially at 55 and 65 °C. This could also be deduced from the NEBI value which is an index of non-enzymatic browning reactions. NEBI was found to be higher in concentrated verjuice at 65 °C (0.18) compared to the samples concentrated at 45 °C (0.14) and 55 °C (0.15) (Table 7.4). Browning occurred because of the increase in temperature, thus the concentration process at 65 °C was a reason for obtaining the darker sample with high browning index (BI). The total color difference in all concentrates produced by the vacuum evaporation method was in the range of the well visible level (ΔE: 3-6) according to Cserhalmi et al. (2006). The most visible color change was observed in the sample produced by the vacuum evaporation at 65 °C/250 mbar (ΔE: 4.30), compared to the evaporation at 45 °C (ΔE: 3.36) and 55 °C (ΔE: 3.58) (Table 7.4). In the literature, it was reported that the browning and degradation of pigments were related to the decrease in lightness and the increase in redness during processing of fruit juices (Assawarachan & Noomhorm, 2010; Maskan, 2006; Rattanathanalerk et al., 2005).

Overall, there was not an important change in color properties between the samples concentrated at 45 °C/913 mbar and 65 °C/763 mbar or at 55 °C/855 mbar and 65 °C/763 mbar even if the boiling temperature and vacuum pressure values were different ($p>0.05$). Therefore, the color difference in the concentrated verjuice samples processed at different conditions can be detected when a higher temperature difference was applied.

7.2.4. Total Phenolic Content of Concentrated Verjuice

Phenolics are the main components responsible from antioxidant capacity of foods (Gurak et al. 2010). It was reported that grape products contain high amount of phytochemical compounds (Capanoglu, de Vos, Hall, Boyacioglu, & Beekwilder, 2013; Castilla et al., 2006). Therefore, the amount of phenolics in the concentrated grape products is an important criterion to evaluate its health effects. Table 7.5 demonstrated the amount of total phenolic content (TPC) of the concentrated verjuice samples produced by different evaporation processes in terms of gram of gallic acid equivalent (GAE) per liter of sample (g GAE/L). The highest amount of phenolics were retained in the concentrated verjuice processed at 45°C/914 mbar (57.15±0.27 g GAE/L), whereas the lowest one was detected in the sample obtained at 100°C and atmospheric pressure (46.43±0.28 g GAE/L). Besides, TPC of the concentrated verjuice obtained by vacuum evaporation at 55 °C/844 mbar was significantly higher (49.05 ±0.82 g GAE/L) than the samples obtained at 65 °C/763 mbar (46.83±1.35 g GAE/L) ($p \leq 0.05$). Figure 7.5 presented the percentage of residual TPC in the concentrated verjuice samples obtained by vacuum and open-pan evaporation methods. According to the Figure, 91.5% of TPC was protected in the concentrated verjuice at the lowest temperature (45 °C/ 914 mbar). However, this percentage was significantly lower, i.e. 78.5%, 75% and 74.3% in the concentrated samples produced at higher temperatures, i.e. for 55 °C, 65 °C and 100 °C, respectively.

Consequently, temperature was an important factor influencing the phenolic content of samples. The amount of TPC in the concentrated verjuice was decreased by increasing the evaporation temperature. Degradation of phenolic compounds by concentration process at high temperatures was previously reported in the literature (Elik, Yanık, Maskan, Gogus, 2016; Hojjatpanah, Fazaeli, & Emam-Djomeh, 2011; Najafabadi et al., 2017). Elik et al. (2016) investigated TPC losses in vacuum and conventional (open-pan) evaporated blueberry concentrates (65 °Brix). They found that the maximum loss of TPC (36.24%) was obtained in the samples subjected to the conventional method. Hojjatpanah et al. (2011) also reported that the reaction rate of the degradation of phenolic matters in black mulberry juice concentrate increased by applying vacuum evaporation at higher temperatures. Najafabadi et al. (2017) determined that TPC degradation in jujube concentrate (65 °Brix) obtained by the conventional heating at 98 °C was higher than that

of obtained by vacuum evaporation at 40 °C. Larrauri, Ruperez, and Saura-Calixto (1997) found that processing temperature at 60 °C did not influence the phenolic content of grape juice, whereas the temperature of 100 °C resulted in 18% degradation of total polyphenolic content. The thermal degradation of phenolic compounds was explained by Maillard and Berset (1995) with three mechanisms, i.e. liberation of the bound phenolics, liberation of the phenolic acid derivatives by partial destruction of lignin, and thermal decomposition of phenolic compounds (Larrauri et al., 1997). Besides, the decrease in the amount of phenolics in concentrated verjuice samples could be also related to browning reactions occurring at high temperatures leading to the destruction of the color pigments (Rattanathanalerk et al., 2005).

Table 7.5. The amount of TPC of concentrated verjuice produced by different evaporation processes

Evaporation Process	TPC (g GAE/ L)
45 °C/914 mbar	57.15 ±0.27a
55 °C/855 mbar	49.05 ±0.82b
65 °C/763 mbar	46.83 ±1.35c
100 °C (CE)	46.43 ±0.28c

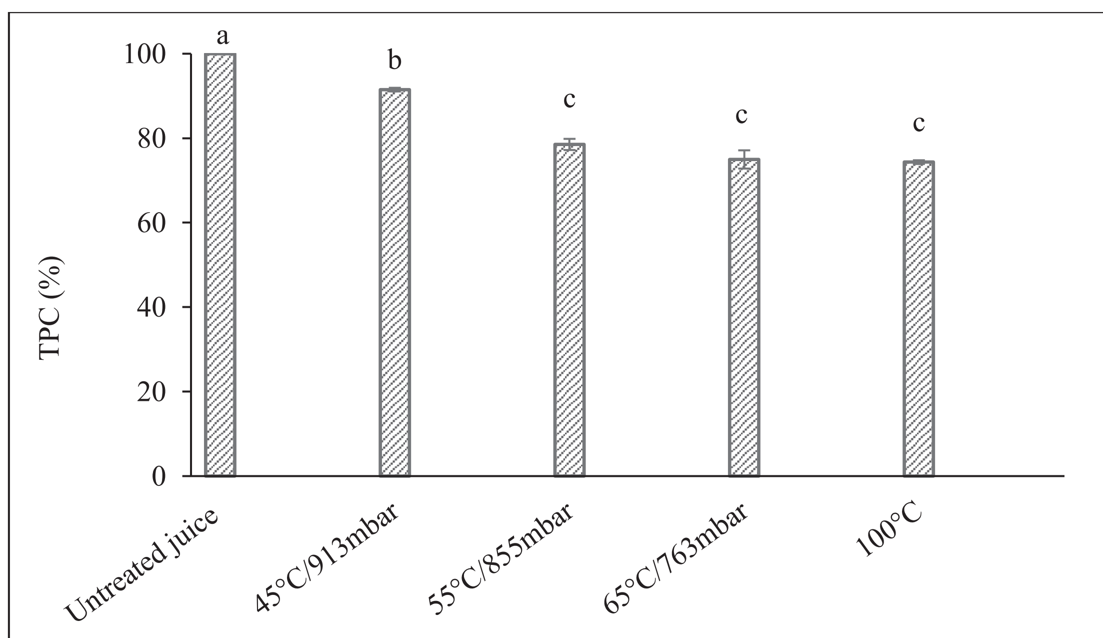


Figure 7.5. The change in the amount of TPC of concentrated verjuice* obtained by different evaporation processes (*: reconstituted verjuice with a dilution factor of 14.13)

7.2.5. NEBI and HMF of Concentrated Verjuice

Non-enzymatic browning index (NEBI) which indicates Maillard reactions, caramelization and pigments degradations in fruit juices is a crucial quality criterion. Non-enzymatic browning reactions results in the loss of organoleptic and nutritional content in the concentrated foods after excessive heating or during storage (Beveridge, Franz, & Harrison, 1986; Koca, Burdurlu, & Karadeniz, 2003; Saguy, Kopelman, & Mizrahi, 1978; Toribio & Lozano, 1984). These reactions occur at the maximum rate in the intermediate moisture products such as concentrated foods with a water activity of 0.6-0.7 (Eskin, 1990). Additionally, hydroxylmethlyfurfural (HMF), an intermediate product of the Maillard reactions and caramelization under acidic conditions, is the other parameter for evaluating the level of browning of thermally concentrated foods and the acceptability of them during storage (Kus, Gogus, & Eren, 2005; Lozano, 1991; Rada-Mendoza, Olano, & Villamiel, 2002). The amount of HMF in a food product can give information about its composition as well as its processing and storage conditions. It was reported that HMF is not detected or approximately zero in fresh or untreated fruit juices (Ascar, 1984; Babsky, Toribio, & Lozano, 1986). The maximum amount of HMF allowed in grape molasses is determined as 75 mg/kg by Turkish Food Codex (Turkish Food Codex, 2007). Therefore, the amount of HMF is directly correlated to NEBI value, and used as a quality parameter in the concentrated verjuice.

Figure 7.6 demonstrates NEBI (Figure 7.6a) and the amount of HMF (Figure 7.6b) in concentrated verjuice obtained at different evaporation conditions. It is clearly seen in Figure 7.6 that non-enzymatic browning and HMF formed in open-pan evaporated samples are comparingly high. The dark-colored concentrate was obtained as a result of high level of browning reactions, therefore, temperature was very important to preserve the color of the product. Furthermore, the open-pan concentrated verjuice is not consumable according to Turkısh Food Codex (2007) due to having excessive amount of HMF (220.1 mg/kg) (Figure 7.6b). The findings of this study were in agreement with the HMF concentration of concentrated grape juice obtained by conventional and vacuum evaporation methods studied by Batu (1991). Batu (1991) observed that HMF content in concentrated grape juice obtained by vacuum evaporation method (32.2 ppm) was noticeably lower than the amount in the concentrated sample produced by conventional evaporation method (681.4 ppm). The high amount of HMF could be generated from the

caramelization reactions in highly acidic concentrated verjuice (pH 2.7). Metin (2014) observed HMF content in the range of 91-11486 mg/kg for different pomegranate concentrates. This excessive amount of HMF was due to the low pH of pomegranate juice (pH 3.5) and high temperature used during concentration. It was also reported that the acidic conditions of the foods may promote the caramelization reactions by the degradation of hexoses, rather than Maillard reactions (Teixido, Nunez, Santos, & Galceran, 2011; Metin, 2014).

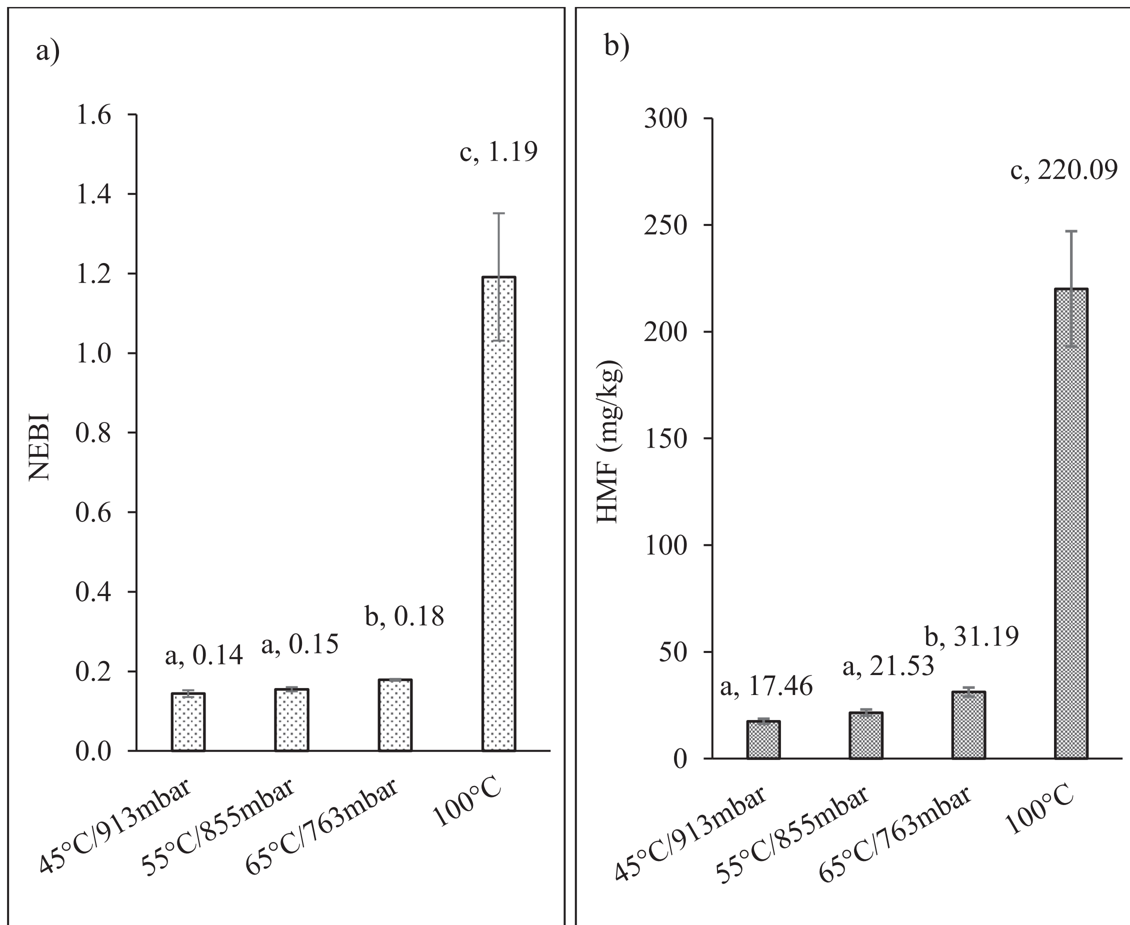


Figure 7.6. Changes in NEBI and the amount of HMF in concentrated verjuice obtained by different evaporation processes (a: NEBI, b:HMF)

Considering the vacuum evaporation method, NEBI and HMF formation were similar in concentrated verjuices obtained at 45 °C/914 mbar and 55 °C/855 mbar, whereas, the values were higher in the sample concentrated at 65 °C/763 mbar (Figure 7.6a and 7.6b). All concentrates could be safely consumed immediately after the vacuum evaporation since the HMF amounts were under the Turkish Food Codex limits (max. 75 mg/kg). Furthermore, the concentrated verjuice samples obtained at 45 °C/914 mbar and

55 °C/855 mbar were the first quality product (max. 25 mg/kg) according to Turkish Standards (TS, 1983).

In this study, the HMF amounts in the concentrated verjuice processed by vacuum evaporation method was higher in comparison with other concentrated products reported in the literature (Bahceci et al., 2015; Kus, Gogus, & Eren, 2005). The reason of this may be correlated with the acidic composition of the verjuice. The HMF amounts in concentrated verjuice samples at 55 °C (21.53 mg/kg) were higher than the amount of HMF observed in tomato juice concentrate (pH 4.1) (7.14 mg/kg) obtained at the vacuum temperature of 70 °C reported by Bahceci et al. (2015). Kus et al. (2005) indicated that the HMF content of pomegranate concentrate with a pH of 3.5 (2476 mg/kg) was much higher than the grape concentrate with a pH of 5.5 (18.4 mg/kg). Therefore, acidic content of the fruit juice can promote the formation of HMF in the concentrated products.

Based on the results of all quality parameters of concentrated verjuice, the best product having high quality was selected as the concentrated verjuice obtained at 45 °C/914 mbar, with minimum color change, lower HMF content and NEBI value, as well as with high phenolic content. The quality parameters of this concentrated verjuice were monitored during the shelf life study.

7.2.6. Changes in the Quality of Concentrated Verjuice during Shelf Life

The change in the quality parameters of the vacuum evaporated verjuice obtained at 45 °C/913 mbar during storage were evaluated (Table 7.6). pH, total soluble solid content, titratable acidity, water activity, moisture content, density and viscosity did not significantly change after 12 weeks of storage at refrigerated (4 °C) and room temperature (25 °C) conditions ($p>0.05$) (Table 7.6). The stability of the concentrated verjuices may be attributed to the constant soluble solid content (approx. 65 °Brix) which stayed unchanged until the end of the storage. Acidity and pH of the concentrated verjuice were similar under two different storage conditions. However, a slight but insignificant decrease in pH and acidity was detected at the end of the 12 weeks. The reason for this decline in pH could be due to the browning reactions catalysing the organic acids and reducing their amounts during storage (Babsky et al. 1986; Lewis, Esselen, & Fellers, 1949). Boranbayeva et al. (2014) observed no changes in pH, acidity and soluble solid

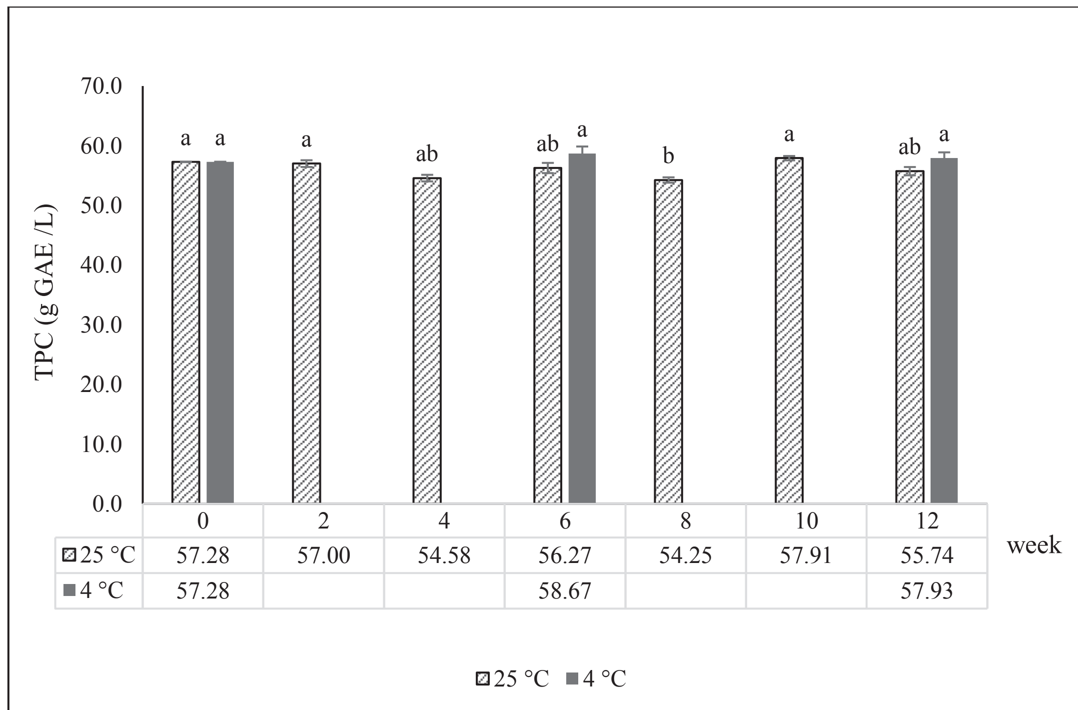
content of black mulberry concentrate during 8 months of refrigerated (5 °C) and room (20 °C) storage conditions. Viscosity of the Newtonian fluids is independent of temperature; therefore, the viscosity of the concentrated verjuice did not change during 12 weeks due to constant storage temperatures (4 °C, 25 °C). Although the viscosity of the samples was not significantly different before and after 12 weeks ($p>0.05$), refrigerated storage caused slight increase in the viscosity (0.12 Pa.s) compared to room conditions (0.10 Pa.s) (Table 7.6). Ibarz et al. (2009) indicated a direct relationship between total soluble solid content and the viscosity of the samples; they stated that "the more concentrated the juice, the greater the viscosity". In this study, the viscosity of the concentrated verjuices having a similar Brix content was constant during storage.

Table 7.6. Changes in physical properties of concentrated verjuice after 12 weeks of storage at refrigerated (4 °C) and room (25 °C) conditions

Week	**0**		**12 (4 °C)**		**12 (25 °C)**	
pH	1.44	±0.07a	1.35	±0.04a	1.42	±0.01a
TSS (°Brix)	65.00	±0.14a	64.85	±0.21a	64.55	±0.21a
T.A (%)	54.67	±0.29a	54.43	±0.06a	54.31	±0.00a
aw	0.75	±0.00a	0.75	±0.01a	0.77	±0.01a
moisture (%)	34.22	±0.34a	33.01	±0.62a	34.22	±0.22a
Density (g/cm^3)	1.35	±0.01a	1.34	±0.00a	1.34	±0.00a
Viscosity (Pa.s)	0.11	±0.01a	0.12	±0.00a	0.10	±0.00a

Figure 7.7 shows the change of TPC of concentrated verjuice during 12 weeks at refrigerated (4 °C) and room temperature (25 °C) conditions. The amounts of phenolic compounds in the concentrated verjuice were similar until the end of the 12 weeks of storage at two different conditions ($p>0.05$). However, there was small fluctuations in TPC of the samples stored at room temperature. Increasing or decreasing of the phenolic compounds throughout the storage period may be related to formation of intermediate dark colored products. It was reported that TPC determined by colorimetric Folin-Ciocalteu method increased during storage period based on the formation of brown

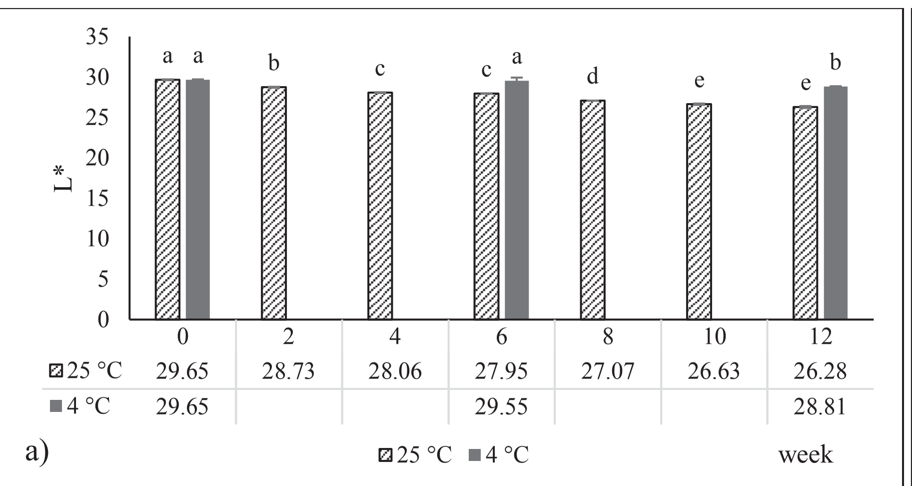
colored intermediate products produced from non-enzymatic browning reactions (Babsky et al. 1986; Spanos, & Wrolstad, 1992). Overall, the TPC of concentrated verjuices remained unchanged throughout storage period.

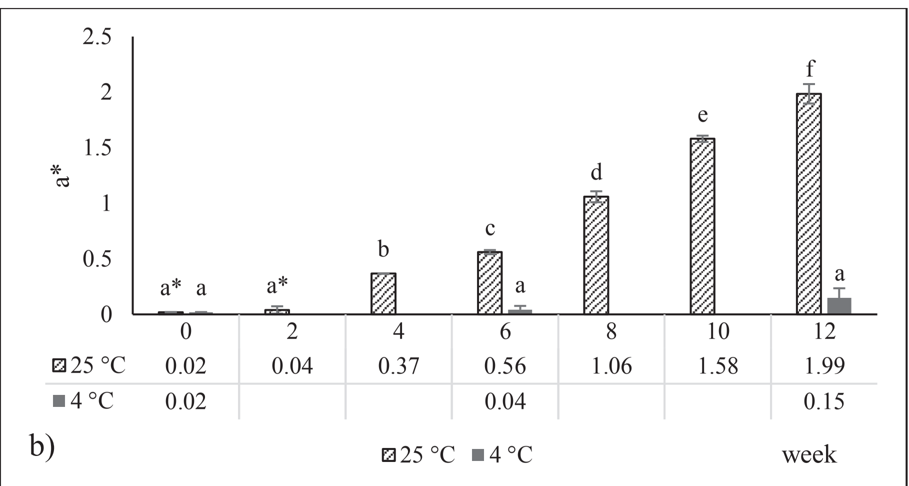


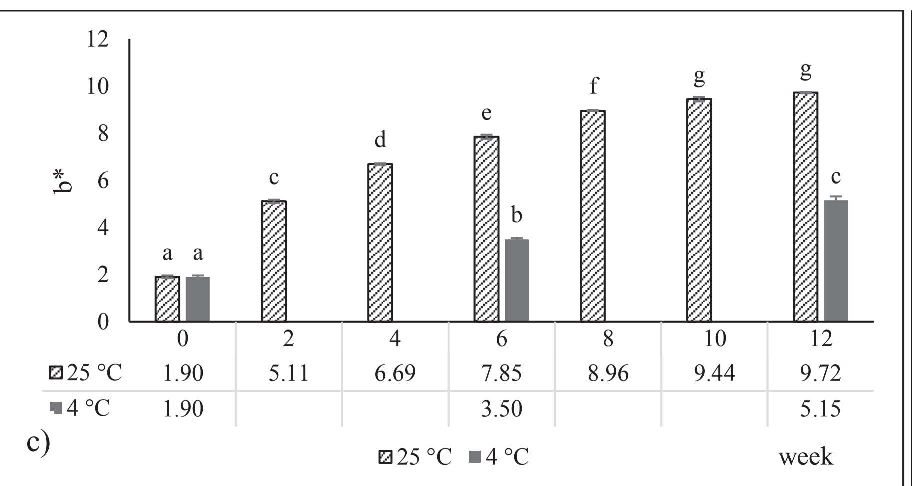
	0	2	4	6	8	10	12
25 °C	57.28	57.00	54.58	56.27	54.25	57.91	55.74
4 °C	57.28			58.67			57.93

Figure 7.7. Changes in TPC of concentrated verjuice stored under refrigerated (4 °C) and room (25 °C) conditions for 12 weeks

Figure 7.8 demonstrates the variations in the color properties (L*, a*, b*, C*, h°, ΔE, BI) of the concentrated verjuice during 12 weeks at refrigerated (4 °C) and room temperature (25 °C). The color of the concentrated verjuice was protected at 4 °C, whereas significant changes occurred at 25 °C during 12 weeks of storage. The L* value of the concentrated samples was almost the same until the end of the 12 weeks of refrigerated storage, whereas it decreased throughout the storage at 25 °C (Figure 7.8a). The most important alterations occurred in a* (redness-greenness) and b* (yellowness-blueness) values in concentrated samples stored at 25 °C. The samples noticeably became redder by the increase of a* values (from 0.02 to 1.99) after 12 weeks at 25 °C. On the other hand, their green color was stable at 4 °C with unchanged a* value (Figure 7.8b). The b* value or yellow color of the concentrates increased nearly 2.5-fold and 5-fold at the end of the 12 weeks of 4 °C and 25 °C storage, respectively (Figure 7.8c).







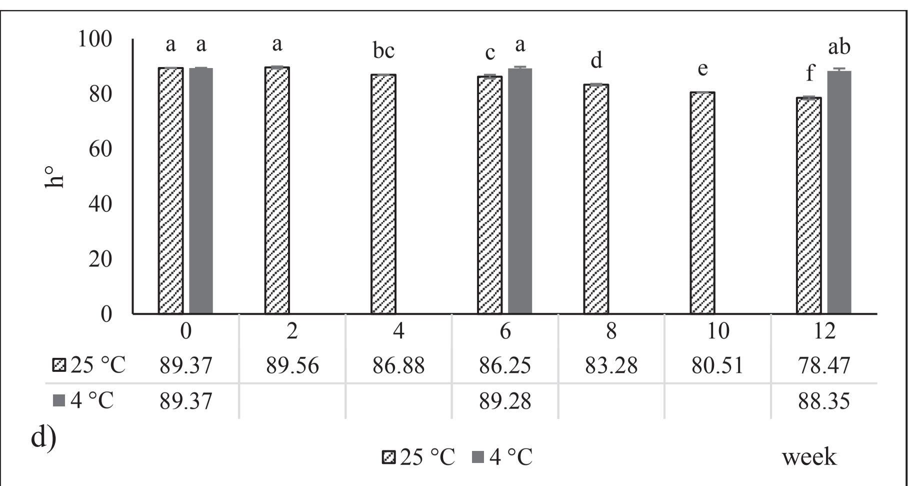
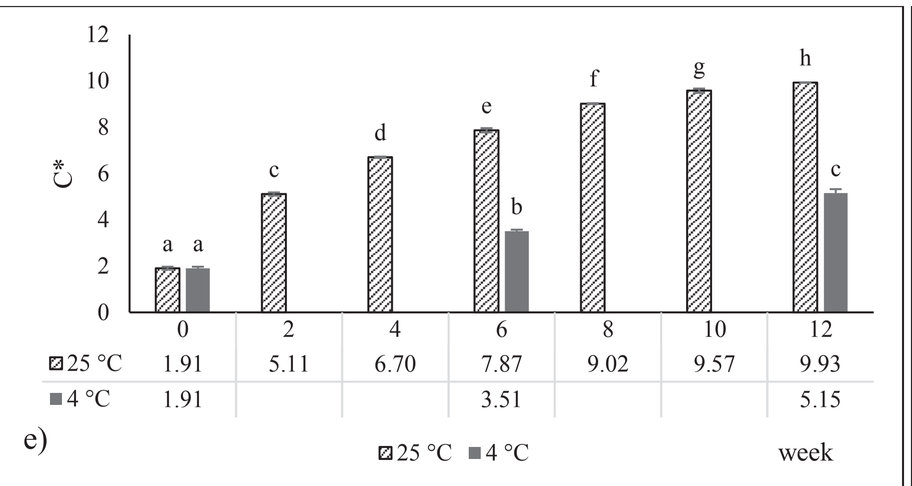
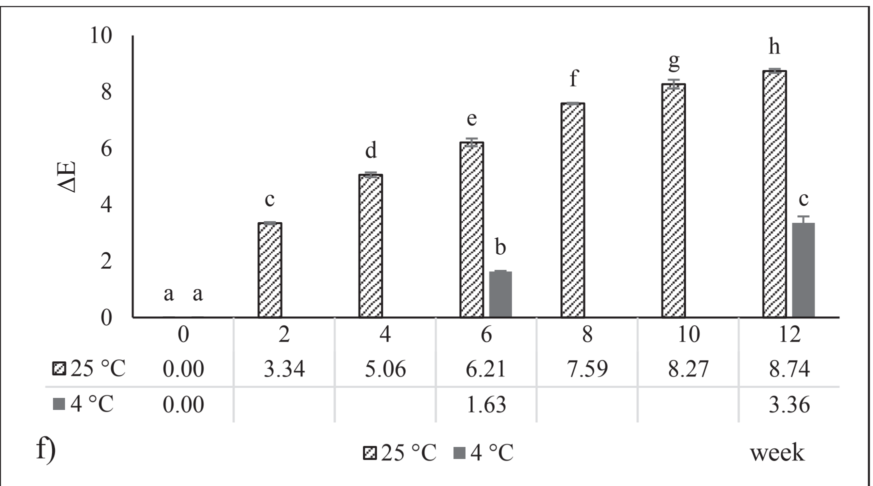


Figure 7.8. Change in color parameters of concentrated verjuice stored in refrigerated (4 °C) and room (25 °C) conditions for 12 weeks (a: lightness, b: redness, c: yellowness, d: hue angle e: Chroma, , f: total color difference, g: browning index)

(Cont. on next page)





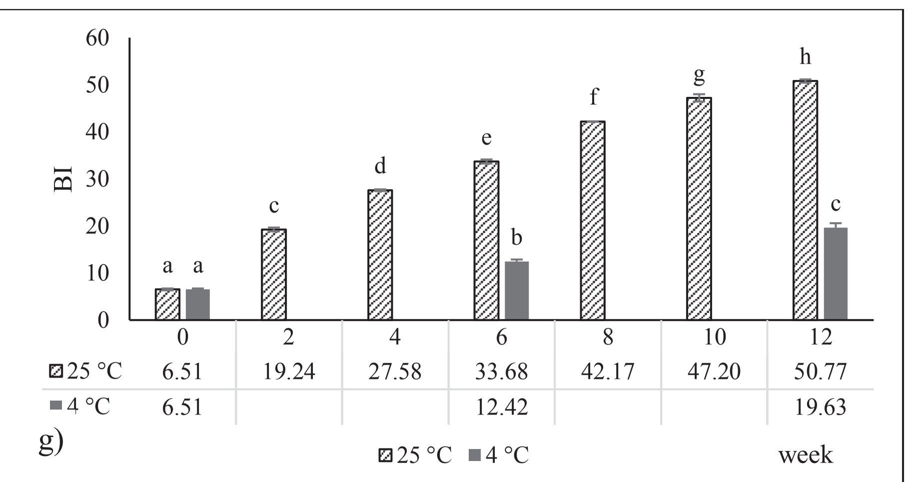


Figure 7.8 (cont.)

Hue angle of the samples were similar when they stored at 4 °C; however, it was slightly decreased at the end of the storage at 25 °C (Figure 7.8d). All samples were in the yellow scale (hue angle of 90°) according to Pathare et al. (2013). The chroma values or color intensity increased considerably throughout 12 weeks at both 4 °C and 25 °C storage conditions (Figure 7.8e). Nevertheless, the change of C* in verjuice was much more noticeable during the storage at room temperature than refrigerated storage. It was noticed from Figure 7.8 that the changes of C* values were highly depended on the changes of b* values of the concentrates. This was due to the yellow color that dominated in the verjuice. Koca et al. (2003) observed a decrease in L* value of the concentrated citrus juices during 8 months of storage at 28, 37 and 45 °C. They also reported lower L* value in the concentrates stored at the higher temperature. Similarly, Burdurlu and Karadeniz (2003) indicated that the decrease of the L* value in apple juice concentrates stored at 20 °C for 16 weeks was in the higher ratio than those stored at 5 °C. Tosun (2004) detected lower L* value and hue angle as well as higher a* value, indicating of browning reactions in the concentrated grape juice (Zile pekmezi) at 55 °C throughout the storage. Lee and Chen (1998) monitored the color of concentrated orange juice during 19 weeks of storage at 4, 14 and 24 °C. They found the highest color change due to the increase of b* and C* at the highest storage temperature (24 °C) attributing to the effect of non-enzymatic browning reactions.

The total color difference (ΔE) values in the concentrated verjuice samples were 3.36 (at 4 °C) and 8.74 (at 25 °C), which were classified as noticeable (1.5-3) and great (6-12) level based on Cserhalmi et al. (2006) (Figure 7.8f). The total color change was mainly related to the browning reactions in the concentrates. Figure 7.8g shows that the rate of increase in Browning index (BI) of the samples, stored at 4 °C and 25 °C throughout 12 weeks, was similar to the rate of increase in ΔE value. The ΔE and BI of the concentrates stored for 2 weeks at 25 °C was similar to the samples stored for 12 weeks at 4 °C. Consequently, the storage temperature was crucial for the preservation of the color in concentrated verjuice.

It was reported that the color change or browning of the concentrated fruit juices were increased at higher storage temperatures (Burdurlu & Karadenizli, 2003; Toribio & Lozano, 1984; Tosun, 2004). This change was generated from the non-enzymatic browning, caramelization or pigment degradations (Bozkurt, Gogus, & Eren, 1999; Tosun, 2004). Gollucke et al. (2008) indicated that refrigerated storage of the concentrated juice protected the color characteristics better.

The changes of NEBI and HMF amount of concentrated verjuice, stored at 4 and 25 °C, are demonstrated in Figure 7.9. According to Figure 7.9a, it was concluded that non-enzymatic browning were increased considerably during 12 weeks of storage at 25 °C. Similarly, HMF amount in the sample stored at 25 °C was increased from 8.49 mg/kg to 158.34 mg/kg at the end the 12 weeks (Figure 7.9b). The sample was inconsumable after 6 weeks by exceeding the critical HMF limits of 75 mg/kg mandated by Turkish Food Codex (2007). However, HMF of the concentrated verjuice at refrigerated storage reached to the critical limit after 12 weeks (77.90 mg/kg), i.e. this sample cannot be consumed after this shelf life period. The same amount of HMF was formed after 4 weeks at 25 °C and 12 weeks at 4 °C, i.e. the concentrated verjuice was stable for 4 weeks at 25 °C or for 12 weeks at 4 °C. Therefore, it can be concluded that the increment in HMF and non-enzymatic browning reactions were highly depended on storage temperature.

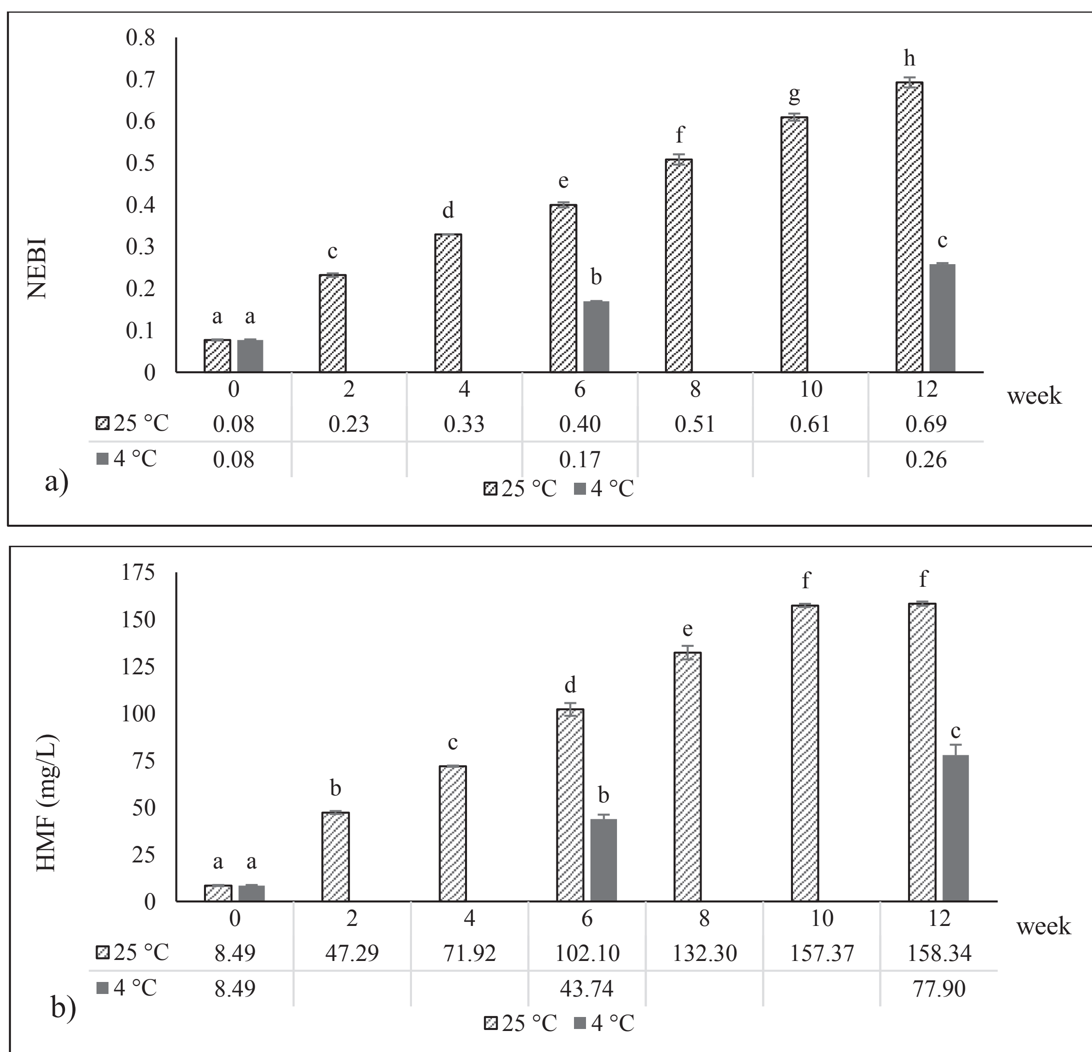


Figure 7.9. Changes in NEBI and the amount of HMF in concentrated verjuice stored in 4 °C and 25 °C for 12 weeks (a: HMF, b: NEBI)

The change of HMF level during storage of the concentrated foods was related to the pH of the samples and storage temperature. Boranbayeva et al. (2014) monitored HMF content of black mulberry concentrate during 8 months storage at 5 and 20 °C. They observed that there was no change in the amount of HMF in black mulberry concentrate (pH: 3.3) during 8 months storage at 5 °C, however, the level of HMF was significantly increased from 93.3 mg/kg to 167.4 mg/kg in the concentrates stored at 20 °C. Tosun and Ustun (2003) studied the HMF changes in white hard grape pekmez (pH 5.5) stored at 20 °C. They reported an increase in HMF content from 9.03 mg/kg to 12.3 mg/kg after 8 months storage. Ozhan (2008) found that HMF formation in concentrate grape juice (Carob pekmez) (pH 5.39). It was raised from 19.6 mg/kg to 32.3 mg/kg after 32 weeks storage at 25 °C. They also reported the reaction rate of HMF formation in the concentrates stored at 35 °C was 14.5 times faster than the one that stored at 25 °C. Therefore, it was found that non-enzymatic browning reactions have important influence on the quality of concentrated products during storage period.

7.2.6.1. Kinetic Modelling of Color Change in Concentrated Verjuice during Shelf Life Period

Variations in color properties of the concentrated verjuice during storage at 4 °C and 25 °C was evaluated by examining changes in each of the color parameters (L*, a*, b*, C*, h°, BI, ΔE, NEBI) with time. Table 7.7 demonstrates the parameters of the zero-order and first-order reaction models applied to the color properties of concentrated verjuice during storage. According to the table, color change of the concentrated verjuice during shelf life was better explained by zero-order model (Table 7.7a) with a higher R^2 for both storage temperatures. The change in a* value at 4 °C was well correlated with the first-order model (higher R^2) (Figure 7.7b). Negative k_0 values of L* value and hue angle implies that lightness of the concentrate was degraded during storage. However, the kinetic constants (k_0) for the other color parameters were positive indicating the formation of colored compounds by non-enzymatic browning reactions (Van Boekel, 2008). Therefore, it can be concluded that the color change of the concentrated verjuice during shelf life was mainly due to Maillard reactions and formation of dark color compounds. Besides, storage temperature affected the rate of the color deteriorations in the concentrate. The most important color change occurred in a* value or redness of the

concentrated product, i.e. reaction rate constant at 25 °C (k_0: 0.17) was 170 times higher than the reaction rate constant at 4 °C (k_0: 0.01). Elik et al. (2017) found the zero-order reaction model was better explained the color change of the blueberry juice than the first-order kinetics. Burdurlu and Karadenizli (2003) studied zero-order modelling for the Maillard reactions at different storage temperatures for 16 weeks. They indicated that the reaction rate constant of apple juice concentrate at 20 °C (k: 0.0014 $week^{-1}$) was much faster than the reaction rate at 4 °C (k: 0.0003 $week^{-1}$). Koca and Burdurlu (2003) also found that zero-order model was the best one to evaluate the change in L* and b* values of citrus juice concentrates during storage at 28, 37 and 45 °C for 8 weeks. It was reported that the color change in the processed fruit products during shelf life was better described by zero-order kinetics (Wibowo, Grauwet, Gedefa, Hendrickx, & Van Loey, 2015).

Table 7.7. Kinetic parameters of zero order (a) and first-order (b) equations for changes in the color of concentrated verjuice during refrigerated and room conditions storage

a)	**25 °C**			**4 °C**		
	k_0 ($week^{-1}$)	**C_0**	**R^2**	**k_0 ($week^{-1}$)**	**C_0**	**R^2**
L*	-0.27	29.41	0.974	-0.07	29.76	0.844
a*	0.17	-0.23	0.951	0.01	0.01	0.880
b*	0.61	3.41	0.890	0.27	1.89	0.999
C*	0.63	3.38	0.900	0.27	1.90	0.999
h	-0.97	90.73	0.955	-0.09	89.51	0.821
BI	3.63	10.67	0.971	1.09	6.29	0.997
ΔE	0.69	1.46	0.917	0.28	-0.02	0.999
NEBI	0.05	0.11	0.990	0.03	0.05	1.000

b)	**25 °C**			**4 °C**		
	k_1 ($week^{-1}$)	**C_0**	**R^2**	**k_1 ($week^{-1}$)**	**C_0**	**R^2**
L*	-0.01	29.43	0.977	0.00	29.76	0.843
a*	0.40	0.03	0.897	0.17	0.02	0.982
b*	0.11	3.20	0.733	0.08	1.97	0.983
C*	0.12	3.19	0.742	0.08	1.98	0.983
h	0.01	90.88	0.951	-0.001	89.51	0.821
BI	0.15	11.19	0.811	0.09	6.72	0.990
ΔE	0.16	1.88	0.771	0.10	0.96	0.988
NEBI	0.16	0.13	0.846	0.20	0.0671	0.970

7.3.Conclusions

In the first part of this chapter, freshly squeezed verjuice was concentrated by using vacuum evaporation method at three different evaporation conditions namely 45 °C/914 mbar, 55 °C/855 mbar and 65 °C/763 mbar, respectively. Open-pan method at 100 °C and atmospheric pressure was also used to compare the quality of the concentrated samples. The removal of water from verjuice, significantly depended on the process temperature and vacuum pressure. Evaporation rate of verjuice was low at low boiling point under vacuum conditions. However, the longest evaporation was carried out by the open-pan method. Physical properties of concentrated verjuice, i.e. pH, acidity, moisture content, water activity, density and viscosity, were not significantly different from each other due to the similar total soluble solid content obtained at the end of the evaporation treatments. Color of the open-pan concentrates was dramatically different from the vacuum evaporated samples in terms of the values of a*(redness-greenness), NEBI and ΔE. Therefore, it was concluded that open-pan method at high temperature caused browning of the juice and resulted in a visible red color in the concentrated samples. The vacuum concentrated verjuice at 45 °C/914 mbar exhibited the most similar color characteristics with the untreated juice. However, the concentrated samples became darker and redder by increasing boiling temperature from 45 °C to 65 °C. Hue angle was decreased at higher temperature of vacuum evaporation, however, saturation of the samples were remained stable due to the similar chroma values. Total phenolic content was decreased by increasing the evaporation temperature. Non-enzymatic browning and HMF production were similar in concentrated verjuice obtained at 45 °C/914 mbar and 55 °C/855 mbar, whereas, the values were higher in the sample concentrated at 65 °C/763 mbar. Furthermore, the conventionally concentrated verjuice cannot be consumed according to the allowed HMF limits by Turkish Food Codex (2007). As a result, when all the quality parameters of concentrated verjuice are compared, the best product with high quality were produced at 45 °C/914 mbar.

In the second part of this chapter, several quality parameters of the concentrated verjuice obtained at 45 °C/914 mbar were evaluated during 12 weeks of storage at refrigerated (4 °C) and room temperature (25 °C). Physical properties and total phenolic content were not changed during 12 weeks at both storage conditions. The fluctuation in the phenolic content of the samples at 25 °C can be due to the non-enzymatic browning

reactions occurred at this temperature. The color of the concentrated verjuice was protected at 4 °C, whereas significant changes occurred at 25 °C during storage. L* value was decreased, samples became darker. The most important alterations occurred in a* (redness-greenness) and b* (yellowness-blueness) at 25 °C. Lower hue angle and higher chroma values were detected at the end of the storage at 25 °C. The increase of total color change and browning index were mainly correlated to the browning reactions occurred in the concentrates. It was concluded that the storage temperature was crucial for the preservation of the color in concentrated verjuice. Non-enzymatic browning and formation of HMF were increased considerably during 12 weeks of storage. The sample was inconsumable at the sixth weeks of storage at 25 °C by exceeding the critical HMF limits of 75 mg/kg mandated by Turkish Food Codex (2007). However, the concentrated verjuice at refrigerated storage was reached to the critical limit after 12 weeks (77.90 mg/kg). Color change in concentrated verjuice during storage was described by zero-order kinetics. Reaction rate constants (k_0) of color parameters indicated that dark colored compounds formed due to non-enzymatic Maillard reactions during storage at 4 °C and 25 °C.

Consequently, concentrated verjuice can be produced in better quality by using vacuum evaporation method employed low boiling temperature. Refrigerated storage temperature should be used in order to maintain the stability of the concentrates during the shelf life period.

CHAPTER 8

CONCLUSION

In this Ph.D. thesis, different koruk (unripe grape) products such as minimally processed verjuice (unripe grape juice), verjuice powder and concentrated verjuice were produced using several processing technologies. Verjuice made from unripe grapes has a specific flavour and generally used as a savoury alternative to vinegar and lemon juice in salads, meals and beverages. By the reason of a limited shelf life of verjuice, long-shelf life verjuice products were obtained and manufacturing technologies were explored in this thesis. Freshly squeezed verjuice was minimally processed by taking US.FDA pasteurization requirement into account. For this purpose, UV-C irradiation and Pulsed UV light (PUV) were assisted by mild heat treatment to achieve 5 log reduction of target microorganism in verjuice. High quality and stable verjuice powder was also produced by using freeze drying method by taking the critical parameters such as moisture content and water activity into account. Maltodextrin was used as a carrier agent to avoid stickiness problem of the powders. Final verjuice product, concentrated verjuice (65 °Brix), was obtained with a minimum quality loss by using vacuum evaporation method. Conventional evaporation at atmospheric pressure (open-pan boiling) was also applied and the product quality was compared to vacuum concentrated verjuice. The quality of verjuice, verjuice powder and concentrated verjuice, were monitored during appropriate storage period at different conditions.

In the verjuice production by using UV-C irradiation, 5D pasteurization conditions were determined. *S. cerevisiae* (NRRL *Y-139*) yeast strain was selected as a target microorganism from the natural fermentation of verjuice. Different initial loads of acid adapted *S. cerevisiae* were inoculated to freshly squeezed verjuice and exposed to UV-C irradiation at 18.1 °C. UV-C treatment was not sufficiently enough to meet 5 log reduction requirement recommended by US.FDA. Therefore, lethal effect of UV-C treatment was increased by combining with mild heating. 5-log reduction of *S. cerevisiae* was succesfully achieved (5.16 log CFU/mL) by applying the UV-C irradiation assisted with mild heating. The juice was exposed to UV-C light for 6.2 min at 77.98 mJ/mL UV dose and 51.24 °C. Inactivation kinetics of acid adapted *S. cerevisiae* in verjuice was

evaluated by using log linear and several non-log linear models. Weibull model was the best fitted model for inactivation data of acid adapted *S. cerevisiae* in verjuice treated with UV-C, mild heating and combined treatments. The synergistic lethal effect of the UV-C irradiation was increased up to 50.79% when it was combined with mild heating at 51.24 °C (Chapter 3).

The verjuice pasteurized by UV-C assisted with mild heating was stored during 12 weeks at refrigerated condition (4.22°C). Additionally, verjuice was pasteurized at 72 °C for 18 s. Untreated and thermally pasteurized verjuice were also used as a positive and negative controls in storage study. Untreated verjuice was completely spoiled by the growth of yeast and molds (3.02 log CFU/ml) at the end of the 10 weeks of storage period. Pasteurized verjuice samples exhibited no microbial growth during 12 weeks of refrigerated storage. Physicochemical properties of verjuice samples, i.e. pH, total soluble solid content and titratable acidity, did not change during shelf life. However, optical properties of verjuice significantly affected during 12 weeks of storage ($p \leq 0.05$) (Chapter 4).

Pulsed-UV light (PUV) was also used for verjuice pasteurization. Different PUV process parameters such as depth of juice layer, distance from the light and number of pulses were investigated. The inactivation efficiency of PUV was increased by decreasing the depth of juice layer, distance the lamp and increasing the number of pulses. When PUV treatment was used alone, it was insufficient for achieving 5D pasteurization of verjuice. Therefore, PUV was combined with different sub-lethal temperatures. The inactivation efficacy of *S. cerevisiae* in the combined treatments was significantly reduced when the depth of verjuice layer were increased at low temperatures. Therefore, the juice depth and temperature were found to be the critical factors in the pasteurization of verjuice by using PUV technology. Pasteruization of verjuice was sufficiently achieved by PUV assisted with mild heating when 3 mm depth of juice layer, 50 pulses (17 J/cm^2, 20 min) at 45 °C and 18 pulses (6.12 J/cm^2, 8.5 min) at 47 °C were used. PUV assisted by mild heat at 47 °C was selected as the pasteurization conditions of verjuice. Quality properties of PUV pasteurized verjuice were monitored during 6 weeks of refrigerated and room temperature storage, together with the positive (pasteurized at 72 °C for 18 s) and negative (no treatment) controls. Untreated juice were completely spoiled by yeasts and moulds (3.70 log CFU/mL) within 2 weeks stored under room conditions (25 °C). No microbial growth occurred in the pasteurized verjuice samples at the end of the 6 weeks under both storage conditions. Although the physicochemical properties of the

verjuice pasteurized with the combined PUV and mild heating treatment did not markedly change, the optical properties were affected during the shelf life. It was recommended that verjuice must be refrigerated to maintain better all the juice quality properties (Chapter 5).

Verjuice powder was produced from freshly squeezed verjuice by using freeze drying method. Different carrier (maltodextrin) concentration and drying times were assessed to determine the optimum conditions. The yield of freeze drying was very high at all conditions. Moisture content and water activity of powders decreased by increasing the maltodextrin (MD) concentration and drying time. All powders were found to be stable except the one obtained using the least MD concentration (10% w/w) and dried for 48 h. Higher MD concentration prevented the browning of verjuice powders. Powder particles showed a skeletal-like structure and were slightly porous. The size of the powder particles decreased by increasing drying time and MD concentration. Freeze drying of the feed mixture having 20% MD for 48 h was selected as the optimum processing conditions. The selected powders were stored for 70 days at the accelerated conditions at 40 °C and 90% RH. Moisture content and water activity of the powder were reached the critical limits, i.e. 4-5%, 0.25, at the end of the 70 days. Verjuice powder was stable, physical powder properties did not change during the accelerated storage. Color of the powder was significantly affected from the storage. The color change occurred upon propagation of browning during the storage period. Powder produced under the selected freeze drying conditions (20%MD, 48h) were in high quality and stable under the accelerated storage conditions.Therefore, it was concluded that powders can be stored for a long time under the conditions of lower temperature and humidity (Chapter 6).

Concentrated verjuice (65 °Brix) was produced by using vacuum evaporation method. Different boiling temperature/vacuum pressure conditions were studied and compared to conventional (open-pan) evaporation. Evaporation rate of verjuice was increased by increasing boiling remperature under vacuum conditions. However, the evaporation rate was the lowest in conventional method applied at the atmospheric pressure. Physical properties of the vacuum and conventionally evaporated verjuice samples were similar. The open-pan evaporated verjuice was dramatically dark color and inconsumable based on its HMF content (>75 mg/kg). The concentrated verjuice obtained at the lowest temparature (45°C/913 mbar) showed similar characteristics compared with the untreated juice, i.e. in terms of color, phenolic content, NEBI and the amount of HMF. As a result, this concentration condition was selected for obtaining the best quality

product and the quality properties of concentrate were monitored during storage at 4 °C and 25 °C for 12 weeks. Physical properties and total phenolic content did not change at both storage conditions. The color of the concentrated verjuice was protected during storage at 4 °C, whereas significant changes occurred at 25 °C storage conditions. The sample stored at 25 °C was inconsumable after 6 weeks and exceeded the critical HMF limits of 75 mg/kg mandated by Turkish Food Codex (2007). However,HMF in the concentrated verjuice stored at refrigerated condition reached to the critical limit after 12 weeks of storage period (77.90 mg/kg). It was concluded that the temperatures at evaporation process and during storage were crucial for preservation of the quality of concentrated verjuice (Chapter 7).

In conclusion, different long shelf life verjuice products such as minimally pasteurized verjuice, verjuice powder and concentrated verjuice were produced in this Ph.D thesis. Verjuice was successfully pasteurized by using nonthermal UV-C or PUV technologies assisted with mild heating. When comparing both technologies, the quality of the pasteurized verjuice with the combined PUV and mild heating was slightly better during storage. However, PUV technology is an expensive method requiring high energy, and it is not commercialized as UV-C irradiation. Thus, UV-C technology is suggested to be more applicable for verjuice production. Quality of the product can be improved by decreasing path length, and providing high flow rate and turbulence in the juice. Stable and high quality verjuice powder was obtained by using freeze drying method. Powder quality can be improved by storing at low temperatures and using a suitable package with a low oxygen permeability. Concentrated verjuice was produced by using vacuum evaporation. Product quality of the concentrates can be improved by processing at lower evaporation temperatures under high vacuum conditions and by storing at refrigerated conditions. As a future study, sensorial analysis of the verjuice products produced in this thesis can be evaluated by consumer tests. Besides, nutritional compositions of these products can be investigated in a future study.

REFERENCES

Abadio, F. D. B., Domingues, A. M., Borges, S. V., & Oliveira, V. M. (2004). Physical properties of powdered pineapple (Ananas comosus) juice-effect of malt dextrin concentration and atomization speed. *Journal of Food Engineering*, *64*(3), 285-287.

Abdelwahed, W., Degobert, G., Stainmesse, S., & Fessi, H. (2006). Freeze-drying of nanoparticles: formulation, process and storage considerations. *Advanced Drug Delivery Reviews*, 58(15), 1688-1713.

Abdul Karim Shah, N. N., Shamsudin, R., Abdul Rahman, R., & Adzahan, N. M. (2016). Fruit juice production using ultraviolet pasteurization: a review. *Beverages*, *2*(3), 22.

Abid, M., Jabbar, S., Hu, B., Hashim, M. M., Wu, T., Wu, Z., ... & Zeng, X. (2014). Synergistic impact of sonication and high hydrostatic pressure on microbial and enzymatic inactivation of apple juice. *LWT-Food Science and Technology*, *59*(1), 70-76.

Acevedo, B. A., Chaves, M. G., Avanza, M. V., & Dellacassa, E. S. (2014). Freeze-drying concentration of R angpur lime juice. *International Journal of Food Science & Technology*, *49*(2), 423-428

Aguilar, K., Ibarz, R., Garvin, A., & Ibarz, A. (2016). Effect of UV-Vis irradiation on enzymatic activities and the physicochemical properties of nectarine juices from different varieties. *LWT-Food Science and Technology*, 65, 969-977.

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.

Aguirre, J. S., Hierro, E., Fernandez, M., & de Fernando, G. D. G. (2014). Modelling the effect of light penetration and matrix colour on the inactivation of *Listeria innocua* by pulsed light. *Innovative Food Science & Emerging Technologies*, *26*, 505-510.

Alimentarius, C. (1981a). Codex standard for concentrated grape juice preserved exclusıvely by physical means (world-wide standard). *Codex Stan*, 83-1981.

Alimentarius, C. (1981b). Codex standard for grape juice preserved exclusively by physical means (world-wide standard). *Codex Stan*, 82-1981.

Alimentarius, C. (2005). General standard for fruit juice and nectar. Codex Standard, 247-2005.

Al-Mutairi, S. K., & Al-Jasser, M. S. (2012). Effect of using Rotary Evaporator on Date Dibs Quality. *Journal of American Science*, *8*(11), 588.

Amazquita, A., Kan-King-Yu, D., & Le Marc, Y. (2011). Modelling microbiological shelf life of foods and beverages. In D. Kilcast & P. Subramaniam (Eds.), *Food and Beverage Stability and Shelf Life* (pp. 405-458). Woodhead Publishing.

Aminian, A., Aminian, B., & Hoseinali, A. N. F. (2006). Effect of unripe grape juice (verjuice) on plasma lipid levels in rabbits rendered hypercholesterolemic by feeding egg yolk. *Acta Medica Iranica*, *44*(4), 230-234.

Aminian, B., Massoompour, S. M., Sadeghalvaad, A., & Omrani, G. H. (2003). Unripe grape juice (verjuice) as a lipid-lowering agent: Fact or fiction. *Archives of Iranian Medicine,* 6, 32-34.

Amiri-Rigi, A., Mohammadifar, M. A., Emam-Djomeh, Z., & Mohammadi, M. (2011). Response surface optimisation of spray dryer operational parameters for low-phenylalanine skim milk powder. *International journal of food science & technology*, *46*(9), 1830-1839.

Andres, L. J., Riera, F. A., & Alvarez, R. (1997). Recovery and concentration by electrodialysis of tartaric acid from fruit juice industries waste waters. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental and Clean Technology*, *70*(3), 247-252.

Aneja, K. R., Dhiman, R., Aggarwal, N. K., Kumar, V., & Kaur, M. (2014). Microbes associated with freshly prepared juices of citrus and carrots. *International journal of food science*, 2014, Article ID 408085.

Anonim. (2003). Verjuice. Retrieved from http://www.eldridge-estate.com.au/verjuice .htm

AOAC. (1990). *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed. Arlington, MA, Washington.

AOAC. 1984. *AOAC Official analytical methods*. Gaithersburg, MD, USA: AOAC International.

Aqualight. (2018). The electromagnetic spectrum [Image]. Retrieved from http://aqualightech.com/UV.asp

Artes, F., & Allende, A.. (2005). Minimal Fresh Processing of Vegetables, Fruits and Juices. *Emerging Technologies for Food Processing*, 26, 676-757.

Artiguez, M. L., Lasagabaster, A., & de Maranon, I. M. (2011). Factors affecting microbial inactivation by pulsed light in a continuous flow-through unit for liquid products treatment. *Procedia Food Science*, *1*, 786-791.

Artik, N., & Velioglu, S. (1992). Meyve suyunun kimyasal bileşimi, işletme ve depolama sırasında değişmesi [Chemical composition of fruit juice, change during operation and storage]. *Meyve suyu endüstrisinde kalite kontrol semineri*, Ankara Üniversitesi Ziraat Fakültesi Gıda Bilimi ve Teknolojisi Bölümü, 9-13 Mart, Ankara.

Ashurst, P. R. (2005). *Chemistry and technology of soft drinks and fruit juices* (Second ed.). John Wiley & Sons.

Assawarachan, R., & Noomhorm, A. (2008). Effect of operating condition on the kinetic of color change of concentrated pineapple juice by microwave vacuum evaporation. *International Journal Food Agricultural and Development*, 6(3&4), 47-53.

Assawarachan, R., & Noomhorm, A. (2010). Changes in color and rheological behavior of pineapple concentrate through various evaporation methods. *International journal of agricultural and biological engineering*, 3(1), 74-84.

Azhu Valappil, Z., Fan, X., Zhang, H. Q., & Rouseff, R. L. (2009). Impact of thermal and nonthermal processing technologies on unfermented apple cider aroma volatiles. *Journal of Agricultural and Food Chemistry*, 57(3), 924-929.

Babsky, N. E., Toribio, J. L., & Lozano, J. E. (1986). Influence of storage on the composition of clarified apple juice concentrate. *Journal of Food Science*, *51*(3), 564-567.

Baeghbali, V., Niakousari, M., & Farahnaky, A. (2016). Refractance Window drying of pomegranate juice: quality retention and energy efficiency. *LWT-Food Science and Technology*, *66*, 34-40.

Bahceci, K. S., Akıllıoglu, H. G., & Gokmen, V. (2015). Osmotic and membrane distillation for the concentration of tomato juice: Effects on quality and safety characteristics. *Innovative Food Science & Emerging Technologies*, *31*, 131-138.

Bahmanyar, A., Asghari, M., & Khoobi, N. (2012). Numerical simulation and theoretical study on simultaneously effects of operating parameters in direct contact membrane distillation. *Chemical Engineering and Processing: Process Intensification*, *61*, 42-50.

Balaswamy, K., Rao, P. P., Nagender, A., Satyanarayana, A. (2011). Preparation of sour grape (Vitis vinifera) beverages and evaluation of their storage stability. *Journal of Food Process Technology,* 2(116), 2.

Bank, H. L., Schmehl, J. L., & Dratch, R. J. (1990). Bacteriocidal Effectiveness of Modulated UV Light. *Applied and Environmental Microbiology*, 56, 3888-3889.

Barbe, A. M., Bartley, J. P., Jacobs, A. L., Johnson, R. A. (1998). Retention of volatile organic flavour/fragrance components in the concentration of liquid foods by osmotic distillation. *Journal of Membrane Science*, 145(1), 67-75.

Barbosa-Canovas, G. V., Pothakamury, U. R., Palou, E., & Swanson, B. G. (1998). *Nonthermal preservation of foods*, 139-161. New York: Marcel Dekker.

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

Barbosa-Canovas, G., Ortega-Rivas, E., Juliano, P., & Yan, H. (2005a). *Food powders: physical properties, processing, and functionality*. Newyork: Kluwer Academic/Plenum Publishers.

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

Barrett, D. M., & Lloyd, B. (2012). Advanced preservation methods and nutrient retention in fruits and vegetables. *Journal of the Science of Food and Agriculture*, *92*(1), 7-22.

Barrett, D. M., Somogyi, L., & Ramaswamy, H. S. (Eds.). (2004). *Processing fruits: science and technology*. United States: CRC press.

Bates, R. P., Morris, J. R. & 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.

Batu, A. (1991). Farklı iki yönteme göre elde edilen kuru üzüm pekmezinin kimyasal bileşiminde oluşan değişmeler üzerinde bir araştırma. *Cumhuriyet Üniv. Tokat Ziraat Fakültesi Dergisi*, *7*(1), 171-178.

Baysal, A. H., Molva, C., & Unluturk, S. (2013). UV-C light inactivation and modeling kinetics of *Alicyclobacillus acidoterrestris* spores in white grape and apple juices. *International Journal of Food Microbiology*, 166(3), 494-498.

Beer, M. (2003). *Cooking with Verjuice*. London: Penguin Books.

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.

Beuchat, L. R. (1982). Thermal inactivation of yeasts in fruit juices supplemented with food preservatives and sucrose. *Journal of Food Science*, *47*(5), 1679-1682.

Beveridge, T., Franz, K., & Harrison, J. E. (1986). Clarified natural apple juice: Production and storage stability of juice and concentrate. *Journal of Food Science*, *51*(2), 411-414.

Bhandari, B. R., & Hartel, R. W. (2005). Phase transitions during food powder production and powder stability.

Bhandari, B. R., Datta, N., & Howes, T. (1997). Problems associated with spray drying of sugar-rich foods. *Drying technology*, 15(2), 671-684.

Bhat, R., & Stamminger, R. (2015). Impact of ultraviolet radiation treatments on the physicochemical properties, antioxidants, enzyme activity and microbial load in freshly prepared hand pressed strawberry juice. *Food Science and Technology International*, *21*(5), 354-363.

Bhavya, M. L., & Umesh Hebbar, H. (2017). Pulsed light processing of foods for microbial safety. *Food Quality and Safety*, *1*(3), 187-202.

Bialka, K. L., & Demirci, A. (2008). Efficacy of pulsed UV-light for the decontamination of *Escherichia coli* O157: H7 and *Salmonella* spp. on raspberries and strawberries. *Journal of Food Science*, *73*(5), M201-M207.

Bigelow, W. D. (1921). The logarithmic nature of thermal death time curves. *The Journal of Infectious Diseases*, 528-536.

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(6), 637-645.

Boranbayeva, T., Karadeniz, F., & Yılmaz, E. (2014). Effect of storage on anthocyanin degradation in black mulberry juice and concentrates. *Food and Bioprocess Technology*, *7*(7), 1894-1902.

Borbalan, A. M. A., Zorro, L., Guillen, 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.

Bozkir, H., & Baysal, T. (2017). Concentration of apple juice using a vacuum microwave evaporator as a novel technique: Determination of quality characteristics. *Journal of Food Process Engineering*, *40*(5).

Bozkurt, H., Gogus, F., & Eren, S. (1999). Nonenzymic browning reactions in boiled grape juice and its models during storage. *Food Chemistry*, *64*(1), 89-93.

Breda, C. A., Sanjinez-Argandona, E. J., & de AC Correia, C. (2012). Shelf life of powdered Campomanesia adamantium pulp in controlled environments. *Food Chemistry*, *135*(4), 2960-2964.

Broihier, K. (1999). The Phytochemical Renaissance. *Food Processing*, Nov. 44,46,48.

Burdurlu, H. S., & Karadeniz, F. (2003). Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry*, *80*(1), 91-97.

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

Cabral, R. A. F., Telis-Romero, J., Telis, V. R. N., Gabas, A. L., & Finzer, J. R. D. (2007). Effect of apparent viscosity on fluidized bed drying process parameters of guava pulp. *Journal of Food Engineering*, *80*(4), 1096-1106.

Caliskan, G., & Dirim, S. N. (2016). The effect of different drying processes and the amounts of maltodextrin addition on the powder properties of sumac extract powders. *Powder Technology*, 287, 308-314.

Caminiti, I. M., Noci, F., Morgan, D. J., Cronin, D. A., & Lyng, J. G. (2012a). The effect of pulsed electric fields, ultraviolet light or high intensity light pulses in combination with manothermosonication on selected physico-chemical and sensory attributes of an orange and carrot juice blend. *Food and bioproducts processing*, *90*(3), 442-448.

Caminiti, I. M., Noci, F., Munoz, A., Whyte, P., Morgan, D. J., Cronin, D. A., & Lyng, J. G. (2011a). Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. *Food Chemistry*, *124*(4), 1387-1392.

Caminiti, I. M., Palgan, I., Munoz, A., Noci, F., Whyte, P., Morgan, D. J., ... & Lyng, J. G. (2012b). The effect of ultraviolet light on microbial inactivation and quality attributes of apple juice. *Food and Bioprocess Technology*, *5*(2), 680-686.

Caminiti, I. M., Palgan, I., Munoz, A., Noci, F., Whyte, P., Morgan, D. J., …& Lyng, J. G. (2011b). The effect of pulsed electric fields (PEF) in combination with high intensity light pulses (HILP) on *Escherichia coli* inactivation and quality attributes in apple juice. *Innovative Food Science & Emerging Technologies*, 12(2), 118-123.

Canitez, N. (2002). Pasteurization of apple cider with UV irradiation. *Master thesis*. The University of Maine.

Cano-Chauca, M., Stringheta, P. C., Ramos, A. M., & Cal-Vidal, J. (2005). Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovative Food Science & Emerging Technologies*, 6(4), 420-428.

Capanoglu, E., de Vos, R. C., Hall, R. D., Boyacioglu, D., & Beekwilder, J. (2013). Changes in polyphenol content during production of grape juice concentrate. *Food Chemistry*, *139*(1-4), 521-526.

Caparino, O. A., Tang, J., Nindo, C. I., Sablani, S. S., Powers, J. R., Fellman, J. K. (2012). Effect of drying methods on the physical properties and microstructures of mango (Philippine 'Carabao'var.) powder. *Journal of Food Engineering*, 111(1), 135-148.

Cardello, A. V. (1994). Consumer expectations and their role in food acceptance. In *Measurement of Food Preferences* (pp. 253-297). Boston, MA: Springer.

Carr, R. L. (1965). Evaluating flow properties of solids. *Chemical Engineering,* 72, 163-168.

Carrillo, M. G., Ferrario, M., & Guerrero, S. (2017). Study of the inactivation of some microorganisms in turbid carrot-orange juice blend processed by ultraviolet light assisted by mild heat treatment. *Journal of Food Engineering*, *212*, 213-225.

Carrillo, M. G., Ferrario, M., & Guerrero, S. (2018). Effectiveness of UV-C light assisted by mild heat on *Saccharomyces cerevisiae* KE 162 inactivation in carrot-orange

juice blend studied by flow cytometry and transmission electron microscopy. *Food Microbiology*.

Casolari, A. (1989). Microbial spoilage of canned fruit juices. In *Biotechnology Applications in Beverage Production* (pp. 65-75). Springer, Dordrecht.

Cassano, A., & Drioli, E. (2007). Concentration of clarified kiwifruit juice by osmotic distillation. *Journal of Food Engineering*, *79*(4), 1397-1404.

Cassano, A., Jioa, B., Drioli, E. (2004). Production of concentrated kiwi fruit juice by integrated membrane process. *Food Research International,* 37, 139-148.

Castilla, P., Echarri, R., Davalos, A., Cerrato, F., Ortega, H., Teruel, J. L., ... & Lasuncion, M. A. (2006). Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. *The American Journal of Clinical Nutrition*, *84*(1), 252-262.

Ceballos, A. M., Giraldo, G. I., & Orrego, C. E. (2012). Effect of freezing rate on quality parameters of freeze dried soursop fruit pulp. *Journal of Food Engineering*, 111(2), 360-365.

Cemeroglu, B. (2004). *Meyve ve Sebze İşleme Teknolojisi*. Ankara: Başkent Klişe Matbaacılık.

Cevik, M., Tezcan, D., Sabanci, S., & Icier, F. (2016). Changes in Rheological Properties of Koruk (Unripe Grape) Juice Concentrates During Vacuum Evaporation. *Akademik Gida*, 14(4), 322-332.

Charles-Rodriguez, A. V., Nevarez-Moorillon, 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.

Chen, G., Campanella, O. H., Corvalan, C. M., & Haley, T. A. (2008). On-line correction of process temperature deviations in continuous retorts. *Journal of Food Engineering*, 84, 258-269.

Chia, S. L., Rosnah, S., Noranizan, M. A. & Wan Ramli, W. D. (2012). The effect of storage on the quality attributes of ultraviolet-irradiated and thermally pasteurized pineapple juices. *International Food Research Journal,* 19 (3), 1001-1010.

Chick, H. (1908). An investigation of the laws of disinfection. *Epidemiology & Infection*, *8*(1), 92-158.

Chisari, M., Barbagallo, R. N., Spagna, G., & Artes, F. (2011). Improving the quality of fresh- cut melon through inactivation of degradative oxidase and pectinase enzymatic activities by UV-C treatment. *International Journal of Food Science & Technology*, 46(3), 463-468.

Chopda, C. A., & Barrett, D. M. (2001). Optimization of guava juice and powder production. *Journal of Food processing and Preservation*, *25*(6), 411-430.

Cisse, M., Vaillant, F., Bouquet, S., Pallet, D., Lutin, F., Reynes, M., & Dornier, M. (2011). Athermal concentration by osmotic evaporation of roselle extract, apple and grape juices and impact on quality. *Innovative Food Science & Emerging Technologies*, *12*(3), 352-360.

Cohen, E., Birk, Y., Mannheim, C. H., & Saguy, I. S. (1998). A rapid method to monitor quality of apple juice during thermal processing. *LWT-Food Science and Technology*, 31(7-8), 612-616.

Crandall, P. G., Chen, C. S., & Carter, R. D. (1982). Models for predicting viscosity of orange juice concentrate. *Food Technology,* 36, 245-252.

Cserhalmi, Z., Sass-Kiss, A., Toth-Markus, M., & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science & Emerging Technologies*, 7(1), 49-54.

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. *CYTA-Journal of Food*, 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.

Devi, K. D., Paul, S. K., & Sahu, J. K. (2016). Study of sorption behavior, shelf life and colour kinetics of vacuum puffed honey powder at accelerated storage conditions. *Journal of food science and technology*, *53*(5), 2334-2341.

Devlieghere, F., Vermeiren, L., & Debevere, J. (2004). New preservation technologies: possibilities and limitations. *International Dairy Journal*, *14*(4), 273-285.

Diamante, L., Umemoto, M. (2015). Rheological properties of fruits and vegetables: a review. *International Journal of Food Properties*, 18(6), 1191-1210.

Dincer, C., Tontul, I., & Topuz, A. (2016). A comparative study of black mulberry juice concentrates by thermal evaporation and osmotic distillation as influenced by storage. *Innovative Food Science & Emerging Technologies*, *38*, 57-64.

Donahue, D. W., Canitez, N., & Bushway, A. A. (2004). UV inactivation of *E. coli* O157: H7 in apple cider: quality, sensory and shelf-life analysis. *Journal of Food Processing and Preservation*, 28(5), 368-387.

Dunn, J. E., Clark, R. W., Asmus, J. F., Pearlman, J. S., Boyer, K., & Parrichaud, F. (1988). Method and apparatus for preservation of foodstuffs. *US International Patent Application*, No WO, 88, 03369.

Dunn, J., Ott, T., & Clark, W. (1995). Pulsed Light Treatment of Food Packaging. *Food Technology*, 95-98.

Dunne, C. P., & Kluter, R. A. (2001). Emerging non-thermal processing technologies: criteria for success. *Australian Journal of Dairy Technology*, *56*(2), 109.

Dziak, J. J., Coffman, D. L., Lanza, S. T., & Li, R. (2012). Sensitivity and specificity of information criteria. *The Methodology Center and Department of Statistics, Penn State, The Pennsylvania State University*, *16*(30), 140.

Eagri. (2018). Chapter 21: Evaporation-Principles, types of evaporators. Development of e-courses for B.Sc (Agriculture). *Tamil Nadu Agricultural University*, AENG 252, lec10. Retrieved from http://eagri.org/eagri50/AENG252/pdf/lec10.pdf

Eastman, J. E., Moore, C. O., (1984). Cold Water Soluble Granular Starch for Gelled Food Composition. U.S. Patent 4465702.

EFJA. (2017). European Fruit Juice Association - Publications. In (Vol. 2017). Brussels: http://www.aijn.org/.

Elez-Martinez, 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.

Elik, A., Yanık, D. K., Maskan, M., & Gogus, F. (2016). Influence of three different concentration techniques on evaporation rate, color and phenolics content of blueberry juice. *Journal of Food Science and Technology*, *53*(5), 2389-2395.

Erbay, B., & Kucukoner, E. (2008). *Gıda endüstrisinde kullanılan farklı kurutma sistemleri*. Türkiye, 10, 21-23.

Erbay, Z., Koca, N., Kaymak-Ertekin, F., & Ucuncu, M. (2015). Optimization of spray drying process in cheese powder production. *Food and Bioproducts Processing*, *93*, 156-165.

Escobedo-Avellaneda, Z., Velazquez, G., Torres, J. A., & Welti-Chanes, J. (2012). Inclusion of the variability of model parameters on shelf-life estimations for low and intermediate moisture vegetables. *LWT-Food Science and Technology*, *47*(2), 364-370.

Eskin, N. A. M. (1990). Biochemistry of food processing: Browning reactions in foods. In *Biochemistry of foods*, (2nd ed. pp. 240-295): Academic Press.

Falguera, V., Pagan, J., & Ibarz, A. (2011). Effect of UV irradiation on enzymatic activities and physicochemical properties of apple juices from different varieties. *LWT-Food Science and Technology*, *44*(1), 115-119.

FAOSTAT. (2018). Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/faostat/en/#data/TP

Fazaeli, M., Emam-Djomeh, Z., Ashtari, A. K., & Omid, M. (2012). Effect of spray drying conditions and feed composition on the physical properties of black mulberry juice powder. *Food and Bioproducts Processing*, *90*(4), 667-675.

Fazaeli, M., Emam-Djomeh, Z., Omid, M., & Kalbasi-Ashtari, A. (2013a). Prediction of the physicochemical properties of spray-dried black mulberry (Morus nigra) juice using artificial neural networks. *Food and Bioprocess Technology*, 6(2), 585-590.

Fazaeli, M., Hojjatpanah, G., & Emam-Djomeh, Z. (2013b). Effects of heating method and conditions on the evaporation rate and quality attributes of black mulberry (Morus nigra) juice concentrate. *Journal of Food Science and Technology*, 50(1), 35-43.

Fellows, P. J. (2017). *Food processing technology: principles and practice* (4th ed.). Cambridge: Woodhead Publishing Series.

Feng, M., Ghafoor, K., Seo, B., Yang, K., & Park, J. (2013). Effects of ultraviolet-C treatment in Teflon®-coil on microbial populations and physicochemical characteristics of watermelon juice. *Innovative Food Science & Emerging Technologies*, 19, 133-139.

Ferrario, M., & Guerrero, S. (2016). Effect of a continuous flow-through pulsed light system combined with ultrasound on microbial survivability, color and sensory shelf life of apple juice. *Innovative Food Science & Emerging Technologies*, *34*, 214-224.

Ferrario, M., Alzamora, S. M., & Guerrero, S. (2013). Inactivation kinetics of some microorganisms in apple, melon, orange and strawberry juices by high intensity light pulses. *Journal of Food Engineering*, *118*(3), 302-311.

Ferrario, M., Alzamora, S. M., & Guerrero, S. (2015). Study of the inactivation of spoilage microorganisms in apple juice by pulsed light and ultrasound. *Food Microbiology*, *46*, 635-642.

Filtenborg, O., Frisvad, J. C., & Thrane, U. (1996). Moulds in food spoilage. *International Journal of Food Microbiology*, *33*(1), 85-102.

Fine, F., & Gervais, P. (2004). Efficiency of pulsed UV light for microbial decontamination of food powders. *Journal of Food Protection*, 67(4), 787-792.

Fitzpatrick, J. J., Barry, K., Cerqueira, P. S. M., Iqbal, T., O'neill, J., & Roos, Y. H. (2007). Effect of composition and storage conditions on the flowability of dairy powders. *International Dairy Journal*, *17*(4), 383-392.

Franceschinis, L., Salvatori, D. M., Sosa, N., & Schebor, C. (2014). Physical and functional properties of blackberry freeze-and spray-dried powders. *Drying Technology*, *32*(2), 197-207.

Franco, T. S., Perussello, C. A., Ellendersen, L. N., & Masson, M. L. (2016). Effects of foam mat drying on physicochemical and microstructural properties of yacon juice powder. *LWT-Food Science and Technology*, *66*, 503-513.

Franks, F. (1998). Freeze-drying of bioproducts: putting principles into practice. *European Journal of Pharmaceutics and Biopharmaceutics*, 45(3), 221-229.

Fredericks, I. N., du Toit, M., & Krugel, M. (2011). Efficacy of ultraviolet radiation as an alternative technology to inactivate microorganisms in grape juices and wines. *Food Microbiology*, *28*(3), 510-517.

Gabriel, A. A. (2012). Inactivation of *Escherichia coli* O157: H7 and spoilage yeasts in germicidal UV-C-irradiated and heat-treated clear apple juice. *Food Control*, 25(2), 425-432.

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.

Gachovska, T. K., Kumar, S., Thippareddi, H., Subbiah, J., & Williams, F. (2008). Ultraviolet and Pulsed Electric Field Treatments Have Additive Effect on Inactivation of *E. coli* in Apple Juice. *Journal of Food Science*, 73(9), M412-417.

Ganan, M., Hierro, E., Hospital, X. F., Barroso, E., & Fernandez, M. (2013). Use of pulsed light to increase the safety of ready-to-eat cured meat products. *Food Control*, 32(2), 512-517.

Garde-Cerdan, T., Arias-Gil, M., Marselles-Fontanet, A. R., Ancin-Azpilicueta, C., & Martin-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.

Gayan, E., Manas, P., Alvarez, I., & Condon, S. (2013). Mechanism of the synergistic inactivation of *Escherichia coli* by UV-C light at mild temperatures. *Applied and environmental microbiology*, 79(14), 4465-4473.

Gayan, E., Serrano, M. J., Alvarez, I., & Condon, S. (2016). Modeling optimal process conditions for UV-heat inactivation of foodborne pathogens in liquid foods. *Food Microbiology*, *60*, 13-20.

Gayan, E., Serrano, M. J., Monfort, S., Alvarez, I., & Condon, S. (2012). Combining ultraviolet light and mild temperatures for the inactivation of *Escherichia coli* in orange juice. *Journal of Food Engineering*, 113(4), 598-605.

Gayan, E., Torres, J. A., Alvarez, I., & Condon, S. (2014). Selection of process conditions by risk assessment for apple juice pasteurization by UV-heat treatments at moderate temperatures. *Journal of Food Protection*, 77(2), 207-215.

Geeraerd, A. H., Herremans, C. H., & Van Impe, J. F. (2000). Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology,* 59(3), 185-209.

Geeraerd, A. H., Valdramidis, V. P., & Van Impe, J. F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, 102(1), 95-105.

Gejima, Y., Zhang, H., & Nagata, M. (2003). Judgment on level of maturity for tomato quality using L* a* b* color image processing. In *Advanced Intelligent Mechatronics, 2003. AIM 2003. Proceedings. 2003 IEEE/ASME International Conference on* (Vol. 2, pp. 1355-1359). IEEE.

Gerard, K. A., & Roberts, J. S. (2004). Microwave heating of apple mash to improve juice yield and quality. *LWT-Food Science and Technology*, *37*(5), 551-557.

Girard, B., & Fukumoto, L. R. (2000). Membrane processing of fruit juices and beverages: a review. *Critical Reviews in Food Science Nutrition*, *40*(2), 91-157.

Goldenmevye. (2017). Grape juice concentrate. *Techniqual specifications*. Retrieved from https://www.goldenmeyve.com.tr/urunler/meyvesuyukonsantreEn/uzumsuyukonsantresi.pdf

Gollucke, A. P. B., De Souza, J. C., & De Queiroz Tavares, D. E. B. O. R. A. (2008). Sensory stability of Concord and Isabel concentrated grape juices during storage. *Journal of Sensory Studies*, *23*(3), 340-353.

Gomez, P. L., Welti-Chanes, J., & Alzamora, S. M. (2011). Hurdle technology in fruit processing. *Annual Review of Food Science and Technology*, *2*, 447-465.

Gomez-Lopez, V. M., Devlieghere, F., Bonduelle, V., & Debevere, J. (2005). Intense light pulses decontamination of minimally processed vegetables and their shelf-life. International Journal of Food Microbiology, 103(1), 79-89.

Gomez-Lopez, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: a review. *Trends in Food Science & Technology*, *18*(9), 464-473.

Gonzalez-Aguilar, G. A., Villegas-Ochoa, M. A., Martinez-Tellez, M. A., Gardea, A. A., & Ayala-Zavala, J. F. (2007). Improving antioxidant capacity of fresh-cut mangoes treated with UV-C. *Journal of Food Science*, *72*(3).

Goula, A. M., & Adamopoulos, K. G. (2005). Spray drying of tomato pulp in dehumidified air: II. The effect on powder properties. *Journal of Food Engineering*, 66(1), 35-42.

Goula, A. M., & Adamopoulos, K. G. (2010). A new technique for spray drying orange juice concentrate. *Innovative Food Science & Emerging Technologies*, 11(2), 342-351.

Gouma, M., Alvarez, I., Condon, S., & Gayan, E. (2015a). Modelling microbial inactivation kinetics of combined UV-H treatments in apple juice. *Innovative Food Science & Emerging Technologies*, *27*, 111-120.

Gouma, M., Gayan, E., Raso, J., Condon, S., & Alvarez, I. (2015b). Inactivation of spoilage yeasts in apple juice by UV–C light and in combination with mild heat. *Innovative Food Science & Emerging Technologies*, 32, 146-155.

Gram, L., Trolle, G., & Huss, H. H. (1987). Detection of specific spoilage bacteria from fish stored at low (0 C) and high (20 C) temperatures. *International Journal of Food Microbiology*, *4*(1), 65-72.

Green, S., Basaran, N., & Swanson, B. G. (2003). High İntensity Light. In P. Zeuthen & L. Bugh-Surensen (Eds.), *Food Preservation Techniques* (pp. 284-302). Boca Raton: CRC press.

Guerrero-Beltran, J. A., & Barbosa-Canovas, G. V. (2004). Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, *10*(3), 137-147.

Guhr, G., & LaChance, P. A. (1997). Role of Phytochemicals in Chronic Disease Prevention.

Gurak, P. D., Cabral, L. M., Rocha-Leao, M. H. M., Matta, V. M., & Freitas, S. P. (2010). Quality evaluation of grape juice concentrated by reverse osmosis. *Journal of Food Engineering,* 96(3), 421-426.

Hammami, C., & Rene, F. (1997). Determination of freeze-drying process variables for strawberries. *Journal of Food Engineering*, 32(2), 133-154.

Harnkarnsujarit, N., Charoenrein, S., & Roos, Y. H. (2012). Microstructure formation of maltodextrin and sugar matrices in freeze-dried systems. *Carbohydrate Polymers*, *88*(2), 734-742.

Hausner, H. H. (1967). Friction conditions in a mass of metal powder. *International Journal of PowderMetallurgy*, 3, 7-13.

Hayoglu, I., Kola, O., Kaya, C., Ozer, S., & Turkoglu, H. (2009). Chemical and sensory properties of verjuice, a traditional 237urkish non-fermented beverage from Kabarcik and Yediveren grapes. *Journal of Food Processing and Preservation*, 33, 252-263.

Hildebrandt, A., & Matchuk, J. (2003). More than ordinary gring. Food Product Design, Weeks Publishing Co., Northbrook, IL. Retrieved from http://www.food productiondesign.com

Hilton, S. T., de Moraes, J. O., & Moraru, C. I. (2017). Effect of sublethal temperatures on pulsed light inactivation of bacteria. *Innovative Food Science & Emerging Technologies*, *39*, 49-54.

Hojjatpanah, G., Fazaeli, M., & Emam-Djomeh, Z. (2011). Effects of heating method and conditions on the quality attributes of black mulberry (Morus nigra) juice concentrate. *International Journal of Food Science & Technology*, 46(5), 956-962.

Huang, Y. J., Tsai, T. Y., & Pan, T. M. (2007). Physiological response and protein expression under acid stress of *Escherichia coli* O157:H7 TWC01 isolated from Taiwan. *Journal of Agricultural and Food Chemistry*, 55, 7182−7191.

Hwang, H. J., Cheigh, C. I., & Chung, M. S. (2015). Relationship between optical properties of beverages and microbial inactivation by intense pulsed light. *Innovative Food Science & Emerging Technologies*, *31*, 91-96.

Ibarz, A., Pagan, J., Panades, R., & Garza, S. (2005). Photochemical destruction of color compounds in fruit juices. *Journal of Food Engineering*, 69(2), 155-160.

Ibarz, R., Falguera, V., Garvin, A., Garza, S., Pagan, J., & Ibarz, A. (2009). Flow behavior of clarified orange juice at low temperatures. *Journal of Texture Studies*, *40*(4), 445-456.

IFST (1999). Development and Use of Microbiological Criteria for Foods. *Institute of Food Science and Technology*, London.

IFST. Institute of Food Science and Technology. (1993). *Shelf life of foods - Guidelines for its determination and prediction*. London: IFTS.

Igual, M., Garcia-Martinez, E., Camacho, M. M. & Martinez-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.

Imram, N. (1999). The role of visual cues in consumer perception and acceptance of a food product. *Nutrition & Food Science*, *99*(5), 224-230.

Islam, M. Z., Kitamura, Y., Yamano, Y., & Kitamura, M. (2016). Effect of vacuum spray drying on the physicochemical properties, water sorption and glass transition phenomenon of orange juice powder. *Journal of Food Engineering*, *169*, 131-140.

Jafari, S. M., Ghalenoei, M. G., & Dehnad, D. (2017). Influence of spray drying on water solubility index, apparent density, and anthocyanin content of pomegranate juice powder. *Powder Technology*, *311*, 59-65.

Jaya, S., Das, H. (2004). Effect of maltodextrin, glycerol monostearate and tricalcium phosphate on vacuum dried mango powder properties. *Journal of Food Engineering*, 63, 125-134.

Jimenez-Sanchez, C., Lozano-Sanchez, J., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2017). Alternatives to conventional thermal treatments in fruit-juice processing. Part 1: Techniques and applications. *Critical Reviews in Food Science and Nutrition*, *57*(3), 501-523.

Jinapong, N., Suphantharika, M., & Jamnong, P. (2008). Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. *Journal of Food Engineering*, *84*(2), 194-205.

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.

Jolivet, S., Arpin, N., Wichers, H.J., & Pellon, G. (1998). Agaricus bisporus browning: a review. *Mycology Research*, 102, 1459-1483.

Juven, B. J., Kanner, J., & Weisslowicz, H. (1978). Influence of orange juice composition on the thermal resistance of spoilage yeasts. *Journal of Food Science*, *43*(4), 1074-1080.

Kadam, D. M., Wilson, R. A., Kaur, S., & Manisha. (2012). Influence of foam mat drying on quality of tomato powder. *International Journal of Food Properties*, *15*(1), 211-220.

Kamffer, Z., Bindon, K. A., & Oberholster, A. (2010). Optimization of a method for the extraction and quantification of carotenoids and chlorophylls during ripening in grape berries (Vitis vinifera cv. Merlot). *Journal of Agricultural and Food Chemistry*, *58*(11), 6578-6586.

Karapinar, M., & Sengun, I. Y. (2007). Antimicrobial effect of koruk (unripe grape-Vitis vinifera) juice against *Salmonella typhimurium* on salad vegetables. *Food Control,* 18, 702-706.

Karel, M., & Lund, D. B. (2003). *Physical principles of food preservation: revised and expanded* (Vol. 129). CRC Press.

Kasper, J. C., & Friess, W. (2011). The freezing step in lyophilization: physico-chemical fundamentals, freezing methods and consequences on process performance and quality attributes of biopharmaceuticals. *European Journal of Pharmaceutics and Biopharmaceutics*, 78(2), 248-263.

Kaya, A., & Belibaglı, K. B. (2002). Rheology of solid gazıantep pekmez. *Journal of Food Engineering*, 54(3), 221-226.

Kaya, A., & Sozer, N. (2005). Rheological behaviour of sour pomegranate juice concentrates (Punica granatum L.). *International Journal of Food Science & Technology*, 40(2), 223-227.

Kaya, Z., & Unluturk , S. (2016). Processing of clear and turbid grape juice by a continuous flow UV system. *Innovative Food Science &Emerging Technologies*, 33, 282-288.

Kaya, Z., Yıldız, S., & Unluturk, S. (2015). Effect of UV-C irradiation and heat treatment on the shelf life stability of a lemon-melon juice blend: multivariate statistical approach. *Innovative Food Science & Emerging Technologies*, 29, 230-239.

Keeney, M., & Bassette, R. (1959). Detection of Intermediate Compounds in the Early Stages of Browning Reaction in Milk Products1. *Journal of Dairy science*, *42*(6), 945-960.

Keyser, M., Muller, 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 & Emerging Technologies*, 9(3), 348-354.

Kha, C. T., Nguyen, M. H., & Roach, P. D. (2010). Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (Momordica cochinchinensis) fruit aril powder. *Journal of Food Engineering*, 98(3), 385-392.

Khan, M. K., Ahmad, K., Hassan, S., Imran, M., Ahmad, N., & Xu, C. (2017). Effect of novel technologies on polyphenols during food processing. *Innovative Food Science & Emerging Technologies*.

Koc, B., Yilmazer, M. S., Balkır, P., & Ertekin, F. K. (2010). Moisture sorption isotherms and storage stability of spray-dried yogurt powder. *Drying Technology*, *28*(6), 816-822.

Koca, N., Burdurlu, H. S., & Karadeniz, F. (2003). Kinetics of nonenzymatic browning reaction in citrus juice concentrates during storage. *Turkish Journal of Agriculture and Forestry*, *27*(6), 353-360.

Koh, P. C., Noranizan, M. A., Karim, R., & Hanani, Z. A. N. (2016). Repetitive pulsed light treatment at certain interval on fresh-cut cantaloupe (Cucumis melo L. reticulatus cv. Glamour). *Innovative Food Science & Emerging Technologies*, *36*, 92-103.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2011). Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. *Australian Journal of Grape and Wine Research*, 17, 230-238.

Koutchma, T. (2008). UV light for processing foods. *Ozone: Science & Engineering*, 30(1), 93-98.

Koutchma, T. N., Forney, L. J., & Moraru, C. I. (2009). *Ultraviolet light in food technology: principles and applications*. CRC press.

Koutchma, T., Keller, S., Chirtel, S., & Parisi, B., (2004). Ultraviolet Disinfection of Juice Products in Laminar and Turbulent Flow Reactors. *Innovative Food Science and Engineering Technologies*, 5, 179-189.

Kramer, B., Wunderlich, J., & Muranyi, P. (2017). Recent findings in pulsed light disinfection. *Journal of Applied Microbiology*, *122*(4), 830-856.

Krishnamurthy, K., Demirci, A., & Irudayaraj, J. M. (2007). Inactivation of *Staphylococcus Aureus* in milk using flow-through pulsed UV-light treatment system. *Journal of Food Science*, *72*(7).

Krokida, M. K., Maroulis, Z. B., & Saravacos, G. D. (2001). The effect of the method of drying on the colour of dehydrated products. *International Journal of Food Science & Technology*, *36*(1), 53-59.

Kumar, P., & Mishra, H. N. (2010). Yogurt powder-A review of process technology, storage and utilization. *Food and Bioproducts Processing*, 2004, 82 (C2), 133-142.

Kus, S., Gogus, F., & Eren, S. (2005). Hydroxymethyl furfural content of concentrated food products. *International Journal of Food Properties*, *8*(2), 367-375.

Kwaw, E., Ma, Y., Tchabo, W., Apaliya, M. T., Sackey, A. S., Wu, M., & Xiao, L. (2018a). Effect of pulsed light treatment on the phytochemical, volatile, and sensorial attributes of lactic-acid-fermented mulberry juice. *International Journal of Food Properties*, *21*(1), 213-228.

Kwaw, E., Tchabo, W., Ma, Y., Apaliya, M. T., Sackey, A. S., Mintah, B. K., ... & Ma, S. (2018b). Effect of storage on quality attributes of lactic-acid-fermented mulberry juice subjected to combined pulsed light and ultrasonic pasteurization treatment. *Journal of Food Measurement and Characterization*, 1-9.

La Cava, E. L., & Sgroppo, S. C. (2015). Evolution during refrigerated storage of bioactive compounds and quality characteristics of grapefruit [*Citrus paradisi* (Macf.)] juice treated with UV-C light. *LWT -Food Science and Technology*, 63(2), 1325-1333.

Lancaster, J. E., Lister, C. E., Reay, P. F., & Triggs, C. M. (1997). Influence of pigment composition on skin color in a wide range of fruit and vegetables. *Journal of the American Society for Horticultural Science*, 122(4), 594-598.

Larrauri, J. A., Ruperez, P., & Saura-Calixto, F. (1997). Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *Journal of Agricultural and Food Chemistry*, *45*(4), 1390-1393.

Lee, H. S., & Chen, C. S. (1998). Rates of Vitamin C Loss and Discoloration in Clear Orange Juice Concentrate during Storage at Temperatures of 4− 24° C. *Journal of Agricultural and Food Chemistry*, *46*(11), 4723-4727.

Lee, K. H., Wu, T. Y., & Siow, L. F. (2013). Spray drying of red (Hylocereus polyrhizus) and white (Hylocereus undatus) dragon fruit juices: physicochemical and antioxidant properties of the powder. *International Journal of Food Science & Technology*, 48(11), 2391-2399.

Lee, W. C., Yusof, S., Hamid, N. S. A., & Baharin, B. S. (2007). Effects of fining treatment and storage temperature on the quality of clarified banana juice. *LWT-Food Science and Technology*, 40(10), 1755-1764.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology*, *55*(1-3), 181-186.

Leistner, L., & Gorris, L. G. (1995). Food preservation by hurdle technology. *Trends in Food Science & Technology*, *6*(2), 41-46.

Leon, K., Mery, D., Pedreschi, F., & Leon, J. (2006). Color measurement in L* a* b* units from RGB digital images. *Food Research International*, 39(10), 1084-1091.

Lewis, V. M., Esselen, W. B., & Fellers, C. R. (1949). Nitrogen-free carboxylic acids in the browning reaction. *Industrial & Engineering Chemistry*, *41*(11), 2591-2594.

Liltved, H., & Landfald, B., (2000). Effects Of High Intensity Light on Ultraviolet-Irradiated and Non-Irradiated Fish Pathogenic Bacteria. *Water Research*, 34(2), 481-486.

Lin, J., Rouseff, R. L., Barros, S., & Naim, M. (2002). Aroma composition changes in early season grapefruit juice produced from thermal concentration. *Journal of Agricultural and Food Chemistry*, *50*(4), 813-819.

Liu, S., Manson, J. E., Lee, I. M., Cole, S. R., Hennekens, C. H., Willett, W. C., & Buring, J. E. (2000). Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. *The American Journal of Clinical Nutrition*, 72(4), 922-928.

Llano, K. R. A., Marselles-Fontanet, A. R., Martin-Belloso, O., & Soliva-Fortuny, R. (2016). Impact of pulsed light treatments on antioxidant characteristics and quality attributes of fresh-cut apples. *Innovative Food Science & Emerging Technologies*, *33*, 206-215.

Lopez-Leiva, M. H. (1988). The use of electrodialysis in food processing. Part 1: Some theoretical concepts. *Lebensmittel-Wissenschaft+ Technologie*, *21*(3), 119-125.

Lozano, J. E. (1991). Kinetics of non enzymatic browning in model systems simulating clarified apple juice. *Lebensmittel-Wissenschaft+ Technologie= Food science+ technology*.

Lozano, J. E., & Ibarz, A. (1997). Colour changes in concentrated fruit pulp during heating at high temperatures. *Journal of Food Engineering*, *31*(3), 365-373.

Maa, Y., Nguyen, P., Sit, K., & Hsu, C. C. (1998). Spray-drying performance of a bench-top spray dryer for protein aerosol powder preparation, *Biotechnology and Bioengineering*, 60, 301-309.

Mafart, P., Couvert, O., Gaillard, S., & Leguerinel, I. (2002). On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *International Journal of Food Microbiology*, 72, 107-113.

Maftei, N. A., Ramos-Villarroel, A. Y., Nicolau, A. I., Martin-Belloso, O., & Soliva-Fortuny, R. (2014). Influence of processing parameters on the pulsed-light inactivation of *Penicillium expansum* in apple juice. *Food Control*, *41*, 27-31.

Maillard, M. N., & Berset, C. (1995). Evolution of antioxidant activity during kilning: Role of insoluble bound phenolic acids of barley and malt. *Journal of Agricultural Food Chemistry,* **1995**, *43*, 1789-1793.

Manas, P., & Pagan, R. (2005). Microbial inactivation by new technologies of food preservation. *Journal of Applied Microbiology*, 98(6), 1387-1399.

Mani, S., Jaya, S., & Das, H. (2002). Sticky issues on spray drying of fruit juices, MBSK, 02-201. In *ASAE/CSAE North-Central Intersectional Meeting*. Saskatoon, SK, Canada.

Mansor, A., Shamsudin, R., Mohd Adzahan, N., & Hamidon, M. N. (2017). Performance of UV pasteurization with quartz glass sleeve on physicochemical properties and microbial activity of pineapple juice. *Journal of Food Process Engineering*, *40*(1).

Manzocco, L., Quarta, B., & Dri, A. (2009). Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. *Innovative Food Science & Emerging Technologies*, *10*(4), 506-511.

Marquenie, D., Geeraerd, A. H., Lammertyn, J., Soontjens, C., Van Impe, J. F., Michiels, C. W., & Nicolaı, B. M. (2003). Combinations of pulsed white light and UV-C or mild heat treatment to inactivate conidia of Botrytis cinerea and Monilia fructigena. *International Journal of Food Microbiology*, *85*(1-2), 185-196.

Marques, L. G., Silveira, A. M., & Freire, J. T. (2006). Freeze-drying characteristics of tropical fruits. *Drying technology*, 24(4), 457-463.

Maskan, M. (2006). Production of pomegranate (Punica granatum L.) juice concentrate by various heating methods: colour degradation and kinetics. *Journal of Food Engineering*, 72(3), 218-224.

Matak, K. E. Churey, J. J., Worobo, R. W., Sumner, S. S., Hovingh, E., Hackney, C. R. & Pierson, M. D. (2005). Efficacy of UV Light for the Reduction of *Listeria Monocytogenes* in Goat's Milk. *Journal of Food Protection*, 68, 2212-2216.

Mazzotta, A. S. (2001). Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157: H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. *Journal of Food Protection*, *64*(3), 315-320.

McBean, D. M., Joslyn, M. A., & Nury, F. S. (1971). Dehydrated fruit. *The Biochemistry of Fruit and Their Products*, 2, 623-652.

McMeekin, T., Bowman, J., McQuestin, O., Mellefont, L., Ross, T., & Tamplin, M. (2008). The future of predictive microbiology: strategic research, innovative applications and great expectations. *International Journal of Food Microbiology*, 128(1), 2-9.

Metin, Z. E. (2014). *Determination of hydroxymethylfurfural levels of pomegranate sour, pomegranate sour sauce and grape molasses sold in Ankara markets* (Master’s thesis). Hacettepe University, Institute of Health Sciences Food Service Systems, Ankara.

Meykon. (2018). Product Specification of Pomegranate Juice Concentrate. Retrieved from http://www.meykon.com/en/urunler/Pomegranate-Juice-Concentrate.pdf

Michalska, A., Wojdylo, A., Lysiak, G. P., & Figiel, A. (2017). Chemical composition an antioxidant properties of powders obtained from different plum juice formulations. *International Journal of Molecular Sciences*, *18*(1), 176.

Miliauskas, G., Venskutonis, P. R., & Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, *85*(2), 231-237.

Miller, F. A., & Silva, C. L. M. (2012). Thermal treatment effects in fruit juices. In S. Rodrigues & F. A. N. Fernandes (Eds.), *Advances in Fruit Processing Technology* (pp. 363-386). Boca Raton: CRC Press.

Miller, R., Jeffrey, W., Mitchell, D., & Elasri, M. (1999). Bacterial Responses to Ultraviolet Light. *ASM News*, 65, 534-541.

Milnefruit. (2018). Pomergranate juice concentrate technical data sheet. Retrieved from https://milnefruit.com/images/specsheets/specifications/Milne_PomegranateJuice32.5BrixSpec.pdf

Mishra, P., Mishra, S., & Mahanta, C. L. (2014). Effect of maltodextrin concentration and inlet temperature during spray drying on physicochemical and antioxidant properties of amla (Emblica officinalis) juice powder. *Food and Bioproducts Processing*, *92*(3), 252-258.

Moßhammer, M. R., Stintzing, F. C., & Carle, R. (2006). Evaluation of different methods for the production of juice concentrates and fruit powders from cactus pear. *Innovative Food Science & Emerging Technologies*, *7*(4), 275-287.

Mousa-Al-Reza, H., Ziba, R., Zakieh, K., rad Tania, S., & Mohammad-Mahdi, S. (2011). The Effect of Verjuice on Serum Lipid Levels in Mice Rendered Atherosclerosis. *Journal of Biologically Active Products from Nature*, *1*(4), 229-235.

Mozowitz, H. J. (1950). Absorption effects in volume irradiation of microorganisms. *Science*, 111(2879), 229-230.

Muller, A., Noack, L., Greiner, R., Stahl, M. R., & Posten, C. (2014). Effect of UV-C and UV-B treatment on polyphenol oxidase activity and shelf life of apple and grape juices. *Innovative Food Science & Emerging Technologies*, 26, 498-504.

Munoz, A., Caminiti, I. M., Palgan, I., Pataro, G., Noci, F., Morgan, D. J., ... & Lyng, J. G. (2012). Effects on *Escherichia coli* inactivation and quality attributes in apple juice treated by combinations of pulsed light and thermosonication. *Food Research International*, *45*(1), 299-305.

Munoz, A., Palgan, I., Noci, F., Morgan, D. J., Cronin, D. A., Whyte, P., & Lyng, J. G. (2011). Combinations of high intensity light pulses and thermosonication for the

inactivation of *Escherichia coli* in orange juice. *Food Microbiology*, *28*(6), 1200-1204.

Murali, S., Kar, A., Mohapatra, D., & Kalia, P. (2015). Encapsulation of black carrot juice using spray and freeze drying. *Food Science and Technology International*, *21*(8), 604-612.

Muzaffar, K., & Kumar, P. (2017). Quality assessment and shelf life prediction of spray dried tamarind pulp powder in accelerated environment using two different packaging materials. *Journal of Food Measurement and Characterization*, *11*(1), 265-271.

NACMCF. (2006). *Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization*. National Advisory Committee on Microbiological Criteria for Foods. Washington, DC.

Najafabadi, N. S., Sahari, M. A., Barzegar, M., & Esfahani, Z. H. (2017). Effects of concentration method and storage time on some bioactive compounds and color of jujube (Ziziphus jujuba var vulgaris) concentrate. *Journal of Food Science and Technology*, *54*(9), 2947-2955.

Nireesha, G. R., Divya, L., Sowmya, C., Venkateshan, N., Babu, M. N., & Lavakumar, V. (2013). Lyophilization/Freeze Drying-An Review. *International Journal of Novel Trends in Pharmaceutical Sciences*, 3(4), 87-98.

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(1), 141-146.

Oberoi, D. P. S., & Sogi, D. S. (2015). Effect of drying methods and maltodextrin concentration on pigment content of watermelon juice powder. *Journal of Food Engineering*, 165, 172-178.

Oms-Oliu, G., Aguilo-Aguayo, I., Martin-Belloso, O., & Soliva-Fortuny, R. (2010a). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (Agaricus bisporus). *Postharvest Biology and Technology*, *56*(3), 216-222.

Oms-Oliu, G., Martin-Belloso, O., & Soliva-Fortuny, R. (2010b). Pulsed light treatments for food preservation. A review. *Food and Bioprocess Technology*, *3*(1), 13.

Oms-Oliu, G., Odriozola-Serrano, I., & Martin-Belloso, O. (2012). The effects of non-thermal technologies on phytochemicals. In *Phytochemicals-A Global Perspective of Their Role in Nutrition and Health*. InTech.

Oncul, N., & Karabiyikli, S. (2015). Factors affecting the quality attributes of unripe grape functional food products. *Journal of Food Biochemistry*, *39*(6), 689-695.

Ozhan, N. B. (2008). *Kinetics of nonenzymatic browning reactions in Carob Pekmez during storage,* (Master thesis). Ankara University, Graduate School of Natural and Applied Sciences Department of Food Science and Technology, Ankara.

Pala, C. U., & Toklucu, A. K. (2011). Effect of UV-C light on anthocyanin content and other quality parameters of pomegranate juice. *Journal of Food Composition and Analysis*, *24*(6), 790-795.

Pala, C. U., & Toklucu, A. K. (2013a). Effects of UV-C light processing on some quality characteristics of grape juices. *Food and Bioprocess Technology*, *6*(3), 719-725.

Pala, C. U., & Toklucu, A. K. (2013b). Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. *LWT-Food Science and Technology*, 50(2), 426-431.

Palgan, I., Caminiti, I. M., Munoz, A., Noci, F., Whyte, P., Morgan, D. J., ... & Lyng, J. G. (2011). Effectiveness of high intensity light pulses (HILP) treatments for the control of *Escherichia coli* and *Listeria innocua* in apple juice, orange juice and milk. *Food Microbiology*, *28*(1), 14-20.

Palomo, E. S., Diaz-Maroto, M. C., Vinas, M. A., Soriano-Perez, A., & Perez-Coello, M. S. (2007). Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. *Food Control*, 18(5), 398-403.

Paniagua-Martinez, I., Ramirez-Martinez, A., Serment-Moreno, V., Rodrigues, S., & Ozuna, C. (2018). Non-thermal Technologies as Alternative Methods for *Saccharomyces cerevisiae* Inactivation in Liquid Media: a Review. *Food and Bioprocess Technology*, 1-24.

Pataro, G., Munoz, A., Palgan, I., Noci, F., Ferrari, G., & Lyng, J. G. (2011). Bacteria inactivation in fruit juices using a continuous flow pulsed light (PL) system. *Food Research International*, 44(6), 1642-1648.

Pataro, G., Sinik, M., Capitoli, M. M., Donsi, G., & Ferrari, G. (2015). The influence of post-harvest UV-C and pulsed light treatments on quality and antioxidant properties of tomato fruits during storage. *Innovative Food Science & Emerging Technologies*, *30*, 103-111.

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(3), 316-320.

Pathare, P. B., Opara, U. L., & Al-Said, F. A. J. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and Bioprocess Technology*, *6*(1), 36-60.

Patras, A., Brunton, N., Da Pieve, S., Butler, F., & Downey, G. (2009). Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. *Innovative Food Science & Emerging Technologies*, *10*(1), 16-22.

Pavan, M. A., Schmidt, S. J., & Feng, H. (2012). Water sorption behavior and thermal analysis of freeze-dried, Refractance Window-dried and hot-air dried açaí (Euterpe oleracea Martius) juice. *LWT-Food Science and Technology*, *48*(1), 75-81.

Peleg, M., & Penchina, C. M. (2000). Modeling microbial survival during exposure to a lethal agent with varying intensity. *Critical Reviews in Food Science and Nutrition*, 40(2), 159-172.

Pena, M. M., Salvia-Trujillo, L., Rojas-Grau, M. A., & Martin-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.

Petruzzi, L., Campaniello, D., Speranza, B., Corbo, M. R., Sinigaglia, M., & Bevilacqua, A. (2017). Thermal Treatments for Fruit and Vegetable Juices and Beverages: A Literature Overview. *Comprehensive Reviews in Food Science and Food Safety*, *16*(4), 668-691.

Przic, D. S., Ruzic, N. L., Petrovic, S. D. (2004). Lyophilization: The process and industrial use. *Hemijska Industrija*, 58(12), 552-562.

Quek, S. Y., Chok, N. K., & Swedlund, P. (2007). The physicochemical properties of spray-dried watermelon powders. *Chemical Engineering and Processing: Process Intensification*, *46*(5), 386-392.

Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for biologists*. Cambridge University Press.

Rada-Mendoza, M., Olano, A., & Villamiel, M. (2002). Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. *Food Chemistry*, *79*(4), 513-516.

Rahn, R. O. (1997). Potassium iodide as a chemical actinometer for 254 nm radiation: Use of iodate as an electron scavenger. *Photochemistry and Photobiology*, 66(4), 450-455.

Ramos-Villarroel, A. Y., Martin-Belloso, O., & Soliva-Fortuny, R. (2011). Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. *European Food Research and Technology*, 233(3), 395-402.

Rankine, B.C. (1968). The importance of yeasts in determining the composition and quality of wines. *Vitis*, 7(1): 22-49.

Rao, M. (1999). *Rheology of Fluid and Semisolid Foods*. Aspen Publication: Gaithersburg, MD.

Rao, P. P., Balaswamy, K., Velu, V., Jyothirmayi, T., & Satyanarayana, A. (2009). Products from grapes of low soluble solids and their quality evaluation. *Journal of Food Science and Technology* (Mysore), 46(1), 77-79.

Raso, J., & Barbosa-Canovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. *Critical Reviews in Food Science and Nutrition,* 43(3):265-285.

Raso, J., Pagan, R., Condon, S., & Sala, F. J. (1998). Influence of temperature and pressure on the lethality of ultrasound. *Applied and Environmental Microbiology*, 64 (2), 465-471.

Rattanathanalerk, M., Chiewchan, N., & Srichumpoung, W. (2005). Effect of thermal processing on the quality loss of pineapple juice. *Journal of Food Engineering*, *66*(2), 259-265.

Ratti, C., (2001). Hot air and freeze-drying of high-value foods - a review. *Journal of Food Engineering*, 49, 311-319.

Ray, B. (2004). Control by heat. *Fundamental Food Microbiology*, 32, 455-465.

Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Publication. (2006). *Meyve suyu üretim teknolojisi* [Fruit juice production technology]. Gıda Serisi, 2. Ankara.

Riganakos, K. A., Karabagias, I. K., Gertzou, I., & Stahl, M. (2017). Comparison of UV-C and thermal treatments for the preservation of carrot juice. *Innovative Food Science & Emerging Technologies*, 42, 165-172.

Rivas, A., Rodrigo, D., Martinez, A., Barbosa-Canovas, G. V., & Rodrigo, M. (2006). Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. *LWT-Food Science and Technology*, *39*(10), 1163-1170.

Rizvi, S. S. (Ed.). (2010). *Separation, extraction and concentration processes in the food, beverage and nutraceutical industries*. Elsevier.

Robertson, G. L., & Samaniego, C. M. L. (1986). Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. *Journal of Food Science*, 51(1), 184–187.

Rodriguez-Hernandez, G. R., Gonzalez-Garcia, R., Grajales-Lagunes, A., Ruiz-Cabrera, M. A., & Abud-Archila, M. (2005). Spray-drying of cactus pear juice (Opuntia

streptacantha): effect on the physicochemical properties of powder and reconstituted product. *Drying Technology*, *23*(4), 955-973.

Roos, Y. H. (1995). Phase transitions in foods (1st ed.). San Diego: Academic Press Inc.

Ross, A. I., Griffiths, M. W., Mittal, G. S., & Deeth, H. C. (2003). Combining nonthermal technologies to control foodborne microorganisms. *International Journal of Food Microbiology*, *89*(2-3), 125-138.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of applied microbiology*, *81*(5), 501-508.

Ross, T. (1999). *Predictive food microbiology models in the meat industry*. Meat and Livestock Australia.

Roustapour, O. R., Hosseinalipour, M., & Ghobadian, B. (2006). An experimental investigation of lime juice drying in a pilot plant spray dryer. *Drying Technology*, 24, 181-188.

Saguy, I. & Karel, M. (1987). Index of deterioration and simulation of quality losses. In: J. G. Kapsalis (Ed.), *Objective Methods in Food Quality Assessment* (pp. 233-260). Boca Raton, FL: CRC.

Saguy, I., Kopelman, I. J., & Mizrahi, S. (1978). Extent of nonenzymatic browning in grapefruit juice during thermal and concentration processes: kinetics and prediction. *Journal of Food Processing and Preservation*, *2*(3), 175-184.

Said, N. E. B., Federighi, M., Bakhrouf, A., & Orange, N. (2010). Effectiveness of pulsed ultraviolet-light treatment for bacterial inactivation on agar surface and liquid medium. *Foodborne Pathogens and Disease*, *7*(11), 1401-1406.

Saikia, S., Mahnot, N. K., & Mahanta, C. L. (2014). Effect of Spray Drying of Four Fruit Juices on Physicochemical, Phytochemical and Antioxidant Properties. *Journal of Food Processing and Preservation*.

Salinas-Roca, B., Soliva-Fortuny, R., Welti-Chanes, J., & Martin-Belloso, O. (2016). Combined effect of pulsed light, edible coating and malic acid dipping to improve fresh-cut mango safety and quality. *Food Control*, *66*, 190-197.

Santhirasegaram, V., Razali, Z., George, D. S., & Somasundram, C. (2015). Comparison of UV-C treatment and thermal pasteurization on quality of Chokanan mango (*Mangifera indica L.*) juice. *Food and Bioproducts Processing*, 94, 313-321.

Sauer, A., & Moraru, C. I. (2009). Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157: H7 in apple juice and apple cider, using pulsed light treatment. *Journal of Food Protection*, *72*(5), 937-944.

Scientific, T. F. (2018). Wine Analysis: from 'Grape to Glass'. *Thermofisher scientific brochures*. Retrieved from https://tools.thermofisher.com/content/sfs/ brochures /XX-72102-Wine-Analysis - XX72102-EN.pdf

Seiji, M., & Iwashita, S. (1965). Enzyme inactivation by ultraviolet light and protective effect of melanin. *The Journal of Biochemistry*, 57(3), 457-459.

Setorki, M., Asgary, S., Eidi, A., & Rohani, A. H. (2010). Effects of acute verjuice consumption with a high-cholesterol diet on some biochemical risk factors of atherosclerosis in rabbits. *Medical Science Monitor*, *16*(4), BR124-BR130.

Setorki, M., Nazari, B., Asgary, S., Azadbakht, L., & Rafieian-Kopaei, M. (2011). Anti atherosclerotic effects of verjuice on hypocholesterolemic rabbits. *African Journal of Pharmacy and Pharmacology*, *5*(8), 1038-1045.

Shearer, A. E., Mazzotta, A. S., Chuyate, R., & Gombas, D. E. (2002). Heat resistance of juice spoilage microorganisms. *Journal of Food Protection*, *65*(8), 1271-1275.

Shishehgarha, F., Makhlouf, J., & Ratti, C. (2002). Freeze-drying characteristics of strawberries. *Drying Technology*, 20(1), 131-145.

Shrestha, A. K., Ua-Arak, T., Adhikari, B. P., Howes, T., & Bhandari, B. R. (2007). Glass transition behavior of spray dried orange juice powder measured by differential scanning calorimetry (DSC) and thermal mechanical compression test (TMCT). *International Journal of Food Properties*, 10(3), 661-673.

Siebert, K. J. (1999). Effects of protein-polyphenol interactions on beverage haze, stabilization, and analysis. *Journal of Agricultural and Food Chemistry*, *47*(2), 353-362.

Silva, F. V., & Gibbs, P. A. (2012). Thermal pasteurization requirements for the inactivation of *Salmonella* in foods. *Food Research International*, *45*(2), 695-699.

Silva, M. A., Sobral, P. J. A., & Kieckbusch, T. G. (2006). State diagrams of freeze-dried camu-camu (Myrciaria dubia (HBK) Mc Vaugh) pulp with and without maltodextrin addition. *Journal of Food Engineering*, *77*(3), 426-432.

Simha, H. V. V., Sharanakumar, H., Nidoni, U., Ramachandra, C. T., Vendan, K. T., & Prakash, K. V. (2012). Comparitive studies on spray-drying and freeze-drying of pomegranate (punica granatum l.) juice fermented with l.Acidophilus, *International Journal of Food and Nutritional Science*, 1(1), 118-127.

Singh, J., Singh, M., Singh, B., Nayak, M., & Ghanshyam, C. (2017). Comparative analyses of prediction models for inactivation of *Escherichia coli* in carrot juice by means of pulsed electric fields. *Journal of Food Science andTtechnology*, *54*(6), 1538-1544.

Singh, V. K., Mandhyan, B. L., Pandey, S., & Singh, R. B. (2013). Process Development for Spray Drying of Ber (Ziziphus jujube L.) Juice. *American Journal of Food Technology*, 8(3).

Singleton, V. L., Rossi, J. L. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144-158.

Sizer, C. E., & Balasubramaniam, V. M. (1999). New intervention processes for minially processed juices. *Food technology.*

Slavin, J. L., & Lloyd, B. (2012). Health benefits of fruits and vegetables. *Advances in nutrition*, *3*(4), 506-516.

Smelt, J. P. P. M., & Brul, S. (2014). Thermal inactivation of microorganisms. *Critical Reviews in Food Science and Nutrition*, 54(10), 1371-1385.

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

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.

Spanos, G. A., & Wrolstad, R. E. (1992). Phenolics of apple, pear, and white grape juices and their changes with processing and storage. A review. *Journal of Agricultural and Food Chemistry*, *40*(9), 1478-1487.

Stradley, L., & Cook, A. (2000). What's Cooking America. Three Forks Books (ISBN: 1-56044-792-3). Retrieved from https://whatscookingamerica.net/Verjus.htm

Stumbo, C.R. (1973). *Thermobacteriology in Food Processing*. New York: Academic Press.

Tandon, K., Worobo, R. W., Churey, J. J., & Padilla-Zakour, O. I. (2003). Storage quality of pasteurized and UV treated apple cider. *Journal of Food Processing and Preservation*, 27(1), 21-35.

Tang, X. C., & Pikal, M. J. (2004). Design of freeze-drying processes for pharmaceuticals: practical advice. *Pharmaceutical Research*, 21(2), 191-200.

Teixido, E., Nunez, O., Santos, F. J., & Galceran, M. T. (2011). 5-Hydroxymethylfurfural content in foodstuffs determined by micellar electrokinetic chromatography. *Food Chemistry*, *126*(4), 1902-1908.

Telis, V. R. N., & Martinez-Navarrete, N. (2009). Collapse and color changes in grapefruit juice powder as affected by water activity, glass transition, and addition of carbohydrate polymers. *Food Biophysics*, *4*(2), 83-93.

Telis, V. R. N., & Martinez-Navarrete, N. (2010). Application of compression test in analysis of mechanical and color changes in grapefruit juice powder as related to glass transition and water activity. *LWT-Food Science and Technology*, *43*(5), 744-751.

Telis, V. R. N., & Sobral, P. J. A. (2002). Glass transitions for freeze-dried and air-dried tomato. *Food Research International*, 35(5), 435-443.

Tezcan, F., Gultekin-Ozguven, M., Diken, T., Ozcelik, B., & Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, *115*(3), 873-877.

Timmermans, R. A. H., Mastwijk, H. C., Knol, J. J., Quataert, M. C. J., Vervoort, L., Van der Plancken, I., …& 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 & Emerging Technologies*, 12(3), 235-243.

Tiwari, B. K., O'Donnell, C. P., Muthukumarappan, K., & Cullen, P. J. (2009b). Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurised juice. *LWT-Food Science and Technology*, *42*(3), 700-704.

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

Tonon, R. V., Brabet, C., & Hubinger, M. D. (2008). Influence of process conditions on the physicochemical properties of açai (Euterpe oleraceae Mart.) powder produced by spray drying. *Journal of Food Engineering*, 88(3), 411-418

Topuz, A., Feng, H., & Kushad, M. (2009). The effect of drying method and storage on color characteristics of paprika. *LWT-Food Science and Technology*, *42*(10), 1667-1673.

Toribio, J. L., & Lozano, J. E. (1984). Nonenzymatic browning in apple juice concentrate during storage. *Journal of Food Science*, *49*(3), 889-892.

Torkamani, A. E., & Niakousari, M. (2011). Impact of UV-C light on orange juice quality and shelf life. *International Food Research Journal*, 18(4).

Torreggiani, D., & Toledo, R. T. (1986). Influence of sugars on heat inactivation, injury and repair of *Saccharomyces cerevisiae*. *Journal of Food Science*, *51*(1), 211-215.

Tournas, V. H., Heeres, J., & Burgess, L. (2006). Moulds and yeasts in fruit salads and fruit juices. *Food Microbiology*, *23*(7), 684-688.

Tran, M. T. T., & Farid, M. (2004). Ultraviolet treatment of orange juice. *Innovative Food Science & Emerging Technologies*, 5, 495-502.

Turkish Food Codex. (2002). *Microbiological criteria announcement of Turkish Food Codex*. *No: 2001/-19. Official Gazette,* 02.09.2001/ 24511. Agriculture and Village Affairs Ministry, Ankara.

Turkish Food Codex. (2007). Türk Gıda Kodeksi-Üzüm Pekmezi Tebligi, Tebliğ No: 2007/27 (in Turkish).

Turkish Food Codex. (2014). Turkısh Food Codex Communique on fruit juices and certain similar products. *Official gazette*, (29080). Republic of Turkey Ministry of Agriculture and Foresty: Ankara.

Turkish Standards [TS]. (1983). Boiled grape juice. *Turkish Standard Institute,* TS 3792.

Turkstat. (2018a). Turkish Statistical Institute. *Production of fruits, beverage and spice crops (For selected products).* Retrieved from http://www.turkstat.gov.tr/PreTabloArama.do?metod=search&araType=vt

Turkstat. (2018b). Turkish Statistical Institute. *Production and Sales of Industrial Products, 2005 - 2016*. Retrieved from http://www.turkstat.gov.tr/PreTabloArama.do?metod=search&araType=vt

Turtoi, M., & Borda, D. (2013). Ultraviolet light efficacy for microbial inactivation on fruit juices, nectars and apple cider. *Journal of Agroalimentary Processes and Technologies*, *19*(1), 130-140.

U.S. FDA. (1996). CFR-code of federal regulations: 21CFR179.41. Retrieved from http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=179.41/

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

U.S. FDA. (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, 6137-6202.

U.S. FDA. (2004). Guidance for industry: Juice HACCP hazards and controls guidance, Final Guidance, March 3, 2004 (1st ed.). *Federal Register*, U.S. Food and Drug Administration, Washington, DC. Retrieved from http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Juice/ucm072557.htm

Unluturk, S., & Atilgan, M. R. (2014). UV-C Irradiation of Freshly Squeezed Grape Juice and Modeling Inactivation Kinetics. *Journal of Food Process Engineering*, 37(4), 438-449.

Unluturk, S., & Atilgan, M. R. (2015). Microbial Safety and Shelf Life of UV-C Treated Freshly Squeezed White Grape Juice. *Journal of food science*, 80(8).

US. (2009). US 818 Fruit juices and nectars-Specification. *Uganda Standard*.

Vaillant, F., Jeanton, E., Dornier, M., O'Brien, G. M., Reynes, M., & Decloux, M. (2001). Concentration of passion fruit juice on an industrial pilot scale using osmotic evaporation. *Journal of Food Engineering*, *47*(3), 195-202.

Valdramidis, V. P., Bernaerts, K., Van Impe, J. F., & Geeraerd, A. H. (2005). An alternative approach to non-log-linear thermal microbial inactivation: modelling the number of log cycles reduction with respect to temperature. *Food Technology and Biotechnology*, 43(4), 321-327.

Van Boekel, M. A. (1996). Statistical aspects of kinetic modeling for food science problems. *Journal of Food Science*, *61*(3), 477-486.

Van Boekel, M. A. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, 74(1), 139-159.

Van Boekel, M. A. (2008). Kinetic modeling of food quality: a critical review. *Comprehensive Reviews in Food Science and Food Safety*, *7*(1), 144-158.

Van Uden, N., Abranches, P., & Cabeca-Silva, C. (1968). Temperature functions of thermal death in yeasts and their relation to the maximum temperature for growth. *Archiv für Mikrobiologie*, 61(4), 381-393.

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*, *17*(6), 288-291.

Vardin, H., & Yasar, M. (2012). Optimisation of pomegranate (Punica Granatum L.) juice spray-drying as affected by temperature and maltodextrin content. *International Journal of Food Science & Technology*, *47*(1), 167-176.

Verma, A., & Singh, S. V. (2015). Spray drying of fruit and vegetable juices-a review. *Critical Reviews in Food Science and Nutrition*, *55*(5), 701-719.

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*(3), 267-273.

Wang, W. D., & Xu, S. Y. (2007). Degradation kinetics of anthocyanins in blackberry juice and concentrate. *Journal of Food Engineering*, *82*(3), 271-275.

Wang, W., Chen, M., & Chen, G. (2012). Issues in freeze drying of aqueous solutions. *Chinese Journal of Chemical Engineering*, 20(3), 551-559.

Wang, Y., Guo, X., Ma, Y., Zhao, X., & Zhang, C. (2018). Effect of ultrahigh temperature treatment on qualities of watermelon juice. *Food Science & Nutrition*, 6(3), 594-601.

Wang, Y., Zhaoxin, L., Fengxia, L., & Xiaomei, B. (2009). Study on microencapsulation of curcumin pigments by spray drying, *European Food Research and Technology*, 229, 391-396.

Weemaes, C. A., Ooms, V., Van Loey, A. M., & Hendrickx, M. E. (1999). Kinetics of chlorophyll degradation and color loss in heated broccoli juice. *Journal of Agricultural and Food Chemistry*, 47(6), 2404-2409.

Wibowo, S., Grauwet, T., Gedefa, G. B., Hendrickx, M., & Van Loey, A. (2015). Quality changes of pasteurised mango juice during storage. Part II: Kinetic modelling of the shelf-life markers. *Food Research International*, *78*, 410-423.

Wilkowska, A., Ambroziak, W., Czyzowska, A., & Adamiec, J. (2016). Effect of microencapsulation by spray-drying and freeze-drying technique on the antioxidant properties of blueberry (Vaccinium myrtillus) juice polyphenolic compounds. *Polish Journal of Food and Nutrition Sciences*, *66*(1), 11-16.

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

Wong, E., Linton, R. H., & Gerrard, D. E. (1998). Reduction of *Escherichia coli* and *Salmonella senftenbergon* pork skin and pork muscle using ultraviolet light. *Food Microbiology*, *15*(4), 415-423.

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.

Yaakob, M. H. (2012). *The Effects of Process Parameters on Concentration of Pineapple Fruit Juice Via Freeze Concentration Technique* (Doctoral dissertation). Universiti Malaysia Pahang, Malaysia.

Yousefi, S., Emam-Djomeh, Z., Mousavi, S. M. A., & Askari, G. R. (2012). Comparing the effects of microwave and conventional heating methods on the evaporation rate and quality attributes of pomegranate (Punica granatum L.) juice concentrate. *Food and Bioprocess Technology*, *5*(4), 1328-1339.

Yuk, H. G., & Marshall, D. L. (2004). Adaptation of *Escherichia coli* O157:H7 to pH alters membrane lipid composition, verotoxin secretion, and resistance to simulated gastric fluid acid. *Applied and Environmental Microbiology*, 70(6), 3500-3505.

Zhang, C., Trierweiler, B., Li, W., Butz, P., Xu, Y., Rufer, C. E., …& Zhao, X. (2011). Comparison of thermal, ultraviolet-c, and high pressure treatments on quality parameters of watermelon juice. *Food Chemistry,* 126(1), 254-260.

Zhao, J. H., Liu, F., Wen, X., Xiao, H. W., & Ni, Y. Y. (2015). State diagram for freeze-dried mango: freezing curve, glass transition line and maximal-freeze-concentration condition. *Journal of Food Engineering*, *157*, 49-56.

ZibaeeNezhad, M. J., Mohammadi, E., Babaie Beigi, M. A., Mirzamohammadi, F., & Salehi, O. (2012). The effects of unripe grape juice on lipid profile improvement. *Cholesterol*, *2012*.

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.

APPENDIX A

VERJUICE PRODUCTION USING ULTRAVIOLET (UV-C) PASTEURIZATION

Table A.1. Acid adaptation studies of *S. cerevisiae* Y139 for the freshly squeezed verjuice

	Survival microbial load (log cfu/ml)					
Time (h)	**1**	**2**	**3**	**4**	**5**	**6**
0	5.85 ±0.06	5.83 ±0.06	5.08 ±0.05	6.40 ±0.03	4.84 ±0.02	5.56 ±0.05
1	5.83 ±0.04	5.93 ±0.03	4.65 ±0.05	6.27 ±0.01	4.81 ±0.05	-
2	5.41 ±0.01	5.39 ±0.00	4.46 ±0.09	6.09 ±0.00	4.51 ±0.01	5.42 ±0.01
3	-	-	4.41 ±0.15	5.96 ±0.05	4.39 ±0.06	
4	4.76 ±0.04	4.22 ±0.06	4.32 ±0.03	5.69 ±0.04	4.42 ±0.12	5.35 ±0.02
6	4.48 ±0.01	3.33 ±0.14	4.00 ±0.03	5.32 ±0.01	4.29 ±0.37	5.34 ±0.02
8	4.36 ±0.03	2.84 ±0.34	3.79 ±0.05	5.17 ±0.01	4.12 ±0.30	-
10	4.27 ±0.01	2.15±0.21	-	-	-	-
24	3.64 ±0.45	1.24 ±0.34	2.07 ±0.07	4.59 ±0.01	3.09 ±0.14	-

The conditions used for inoculation of *S. cerevisiae* Y139 into the verjuice were;

1: İnitial (viability of the cells in verjuice without acid adaptation): *S. cerevisiae* Y139 from the stock culture was inoculated to the YPD broth without acidification (pH 6.55), incubated at 30 °C and 200 rpm for 24 h in the shaker and then inoculated to verjuice.

2: *S. cerevisiae* Y139 from the stock culture was inoculated to the YPD broth acidified to pH 3.0, incubated at 30 °C and 200 rpm for 24 h in the shaker.

3: *S. cerevisiae* Y139 colonies survived in the verjuice after 72 h were inoculated to the YPD broth acidified to pH 2.70, incubated at 30 °C and 200 rpm for 24 h in the shaker. Brix value of the juice (4%) was also increased to 8.5% with addition of dextrose.

4: *S. cerevisiae* Y139 colonies survived in the verjuice after 72 h was inoculated to the YPD broth acidified to pH 3.50, incubated at 30 °C and 200 rpm for 24 h in the shaker. Brix value of the juice (4%) was also increased to 8.5% with addition of dextrose.

5: *S. cerevisiae* Y139 colonies survived in the verjuice after 72 h was inoculated to the YPD broth acidified to pH 3.50, incubated at 30 °C and 200 rpm for 24 h in the shaker. Then 1 ml of the culture in pH 3.50 was transferred to the YPD broth acidified to pH 2.70, incubated at 30 °C and 200 rpm for 24 h in the shaker.

6: *S. cerevisiae* Y139 colonies survived in the verjuice after 72 h was inoculated to the YPD broth acidified to pH 3.50, incubated at 30 °C and 200 rpm for 24 h in the shaker. Then 1 ml of the culture in pH 3.50 was transferred to the YPD broth acidified to pH 2.70 incubated at 30 °C and 200 rpm for 48 h in the shaker.

Table A.2. Logarithmic reduction data of acid adapted *S. cerevisiae* (NRRL Y-139) by UV-C process at room temperature (20 °C)

UV-C processes (UV)						
	low initial load		high initial load		actinometric UV dose	
cycles	avg	st dev	avg	st dev	avg	st dev
1	0.07	±0.145	0.04	±0.069	0.292	±0.001
2	0.10	±0.082	0.05	±0.037	0.420	±0.000
3	0.16	±0.065	0.09	±0.141	0.767	±0.003
4	0.26	±0.029	0.12	±0.084	1.070	±0.002
5	0.32	±0.047	0.26	±0.092	1.403	±0.002
6	0.41	±0.019	0.30	±0.033	1.646	±0.004
7	0.51	±0.029	0.33	±0.046	2.011	±0.003
8	0.54	±0.016	0.40	±0.032	2.301	±0.002

Table A.3. Logarithmic reduction data of acid adapted *S. cerevisiae* (NRRL Y-139) by mild heating processs (i: MH I, ii: MH2) with the temperature distribution

(i)	Mild Heating Process 1 (MH 1) $T_{juice\ inlet}$: 50 °C & $T_{circulated\ water}$: 55 °C					
	log reductions (log CFU/mL)		Temperature distribution (°C)			
cycle	avg	st dev	sample outlet		sample tank	
1	0.59	±0.19	37.38	±1.00	48.17	±0.66
2	0.67	±0.18	44.52	±0.76	47.65	±0.70
3	0.68	±0.22	45.27	±0.69	48.07	±0.69
4	0.80	±0.35	45.73	±0.52	48.37	±0.59
5	0.71	±0.23	46.15	±0.40	48.60	±0.68
6	0.77	±0.22	46.33	±0.44	48.88	±0.62
7	0.90	±0.31	46.57	±0.35	48.92	±0.51
8	0.96	±0.25	46.70	±0.36	49.10	±0.52

(ii)	Mild Heating Process 2 (MH 2) $T_{juice\ inlet}$: 50 °C & $T_{circulated\ water}$: 60 °C					
	log reductions (log CFU/mL)		Temperature distribution (°C)			
cycle	avg	st dev	sample outlet		sample tank	
1	0.89	±0.24	39.37	±2.64	51.37	±1.06
2	1.31	±0.24	46.75	±1.20	50.58	±1.02
3	1.41	±0.26	47.92	±0.83	51.33	±0.88
4	1.52	±0.39	48.70	±0.56	51.72	±0.75
5	1.81	±0.33	49.12	±0.50	52.13	±0.69
6	2.27	±0.39	49.68	±0.29	52.23	±0.46
7	2.53	±0.26	49.90	±0.28	52.48	±0.58
8	3.13	±0.05	50.08	±0.15	52.92	±0.71

Table A.4. Logarithmic reduction data of acid adapted *S. cerevisiae* (NRRL Y-139) by UV-C combined with mild heating processes (i: UV+MH1 & ii: UV+MH2) with the temperature distribution

(i)	**Hurdle process 1 (UV + MH1)** $T_{juice\ inlet}$: 50 °C & $T_{circulated\ water}$: 55 °C							
	Log reductions (log CFU/mL)		Actinometric UV dose (J/mL)		Temperature distribution (°C)			
cycle	avg	st dev	avg	st dev	Sample outlet		Sample tank	
1	0.612	±0.051	0.205	±0.000	40.90 ±	±0.14	47.75	±0.07
2	0.822	±0.183	0.274	±0.000	46.35 ±	±0.07	48.45	±0.07
3	1.091	±0.235	0.496	±0.002	46.85 ±	±0.07	48.85	±0.07
4	1.438	±0.226	0.687	±0.002	47.45 ±	±0.07	49.40	±0.00
5	1.857	±0.198	0.897	±0.001	47.80 ±	±0.00	49.55	±0.07
6	2.438	±0.327	1.050	±0.002	47.95 ±	±0.07	49.55	±0.07
7	2.839	±0.359	1.281	±0.001	48.10 ±	±0.14	49.60	±0.14
8	3.588	±0.327	1.463	±0.000	48.25 ±	±0.07	49.95	±0.07

(ii)	**(Hurdle process 2 (UV + MH2)** $T_{juice\ inlet}$: 50 °C & $T_{circulated\ water}$: 60 °C							
	log reductions (log CFU/mL)		Actinometric UV dose (J/mL)		Temperature distribution (°C)			
cycle	avg	st.dev.	avg	st.dev.	Sample outlet		Sample tank	
1	1.251	±0.354	0.202	±0.007	42.20	±2.26	50.10	±0.57
2	1.571	±0.105	0.267	±0.000	48.10	±0.07	51.35	±0.64
3	1.895	±0.034	0.481	±0.002	49.30	±0.07	52.55	±0.49
4	2.730	±0.199	0.661	±0.002	50.50	±0.07	52.95	±0.64
5	3.750	±0.100	0.862	±0.002	50.90	±0.07	53.15	±0.64
6	5.163	±0.239	1.006	±0.003	51.35	±0.07	53.45	±0.49
7	-	-	1.225	±0.003	51.65	±0.07	53.45	±0.35
8	-	-	1.400	±0.002	51.75	±0.07	53.40	±0.42

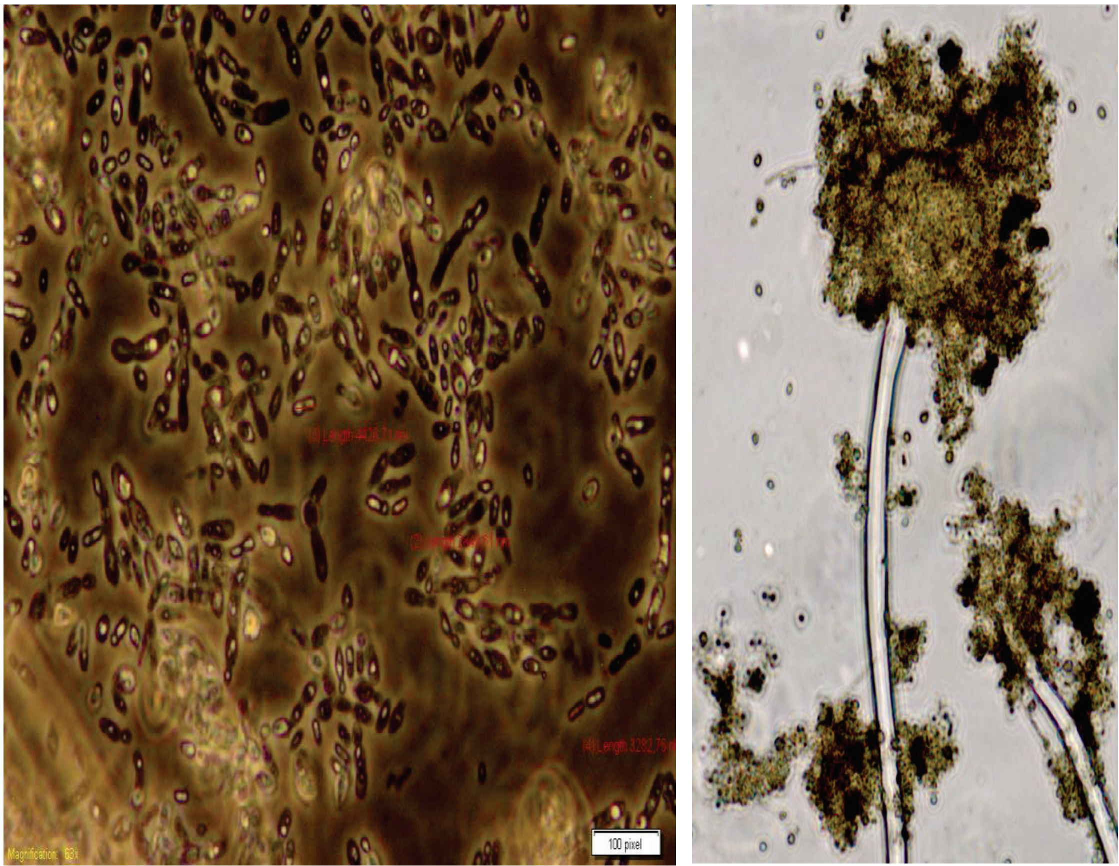


Figure A.1. Microscobic appearance of the microorganisms in naturally fermented verjuice.

Figure A.2. The color of verjuice during 6 cycles in the UV+MH2 process

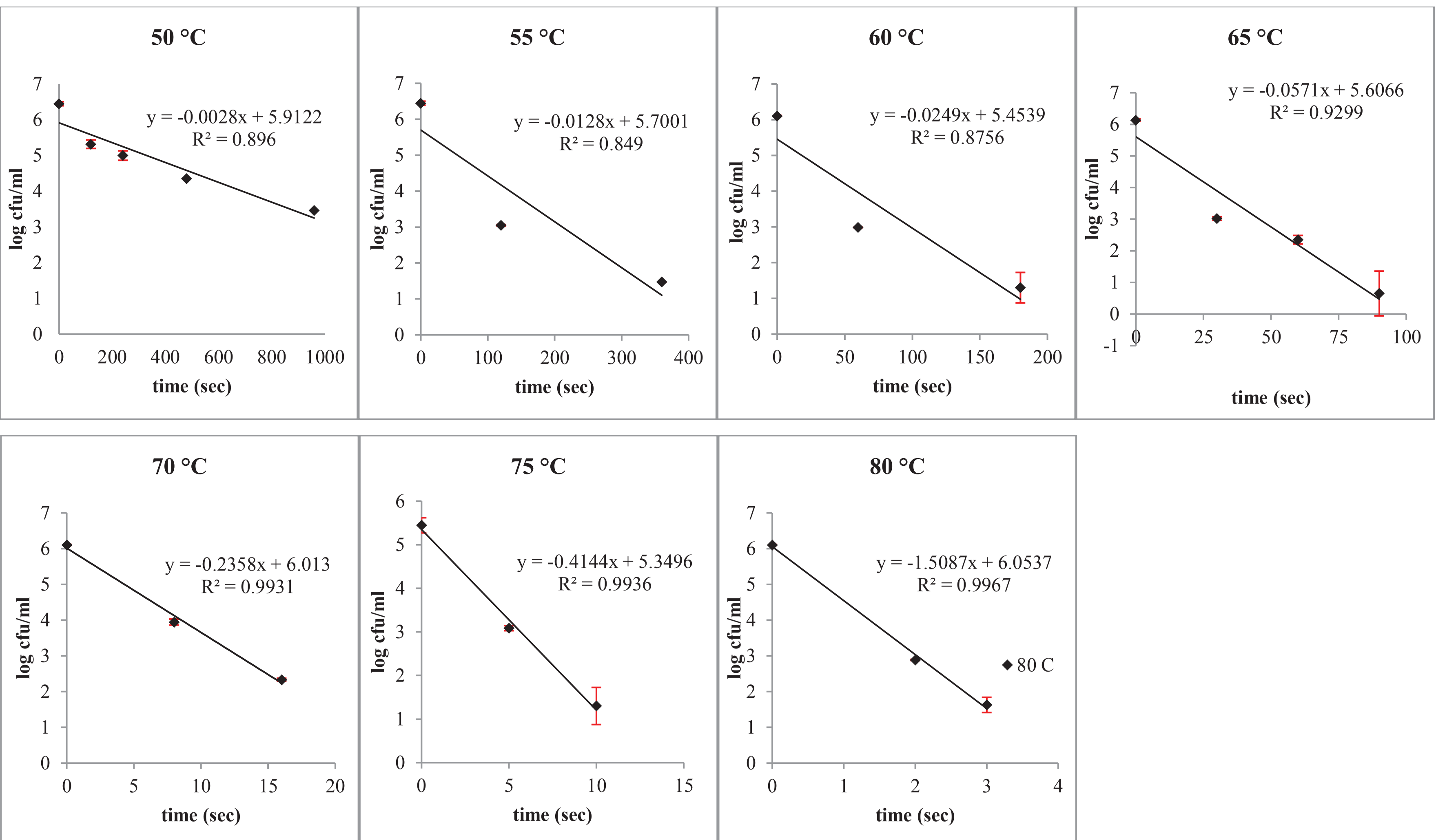


Figure A.3. Thermal Death Time (TDT) curves of *S. cerevisiae* (NRRL Y-139) for different temperatures in freshly squeezed verjuice

Table A.5. D values of acid adapted *S. cerevisiae* (NRRL Y-139) in freshly squeezed verjuice at different temperatures

Temperature (°C)	D value (sec)	R^2 of the Linear Equation
50	361	0.896
55	78.33	0.849
60	40.16	0.8756
65	17.51	0.9299
70	4.24	0.9931
75	2.41	0.9936
80	0.66	0.9967

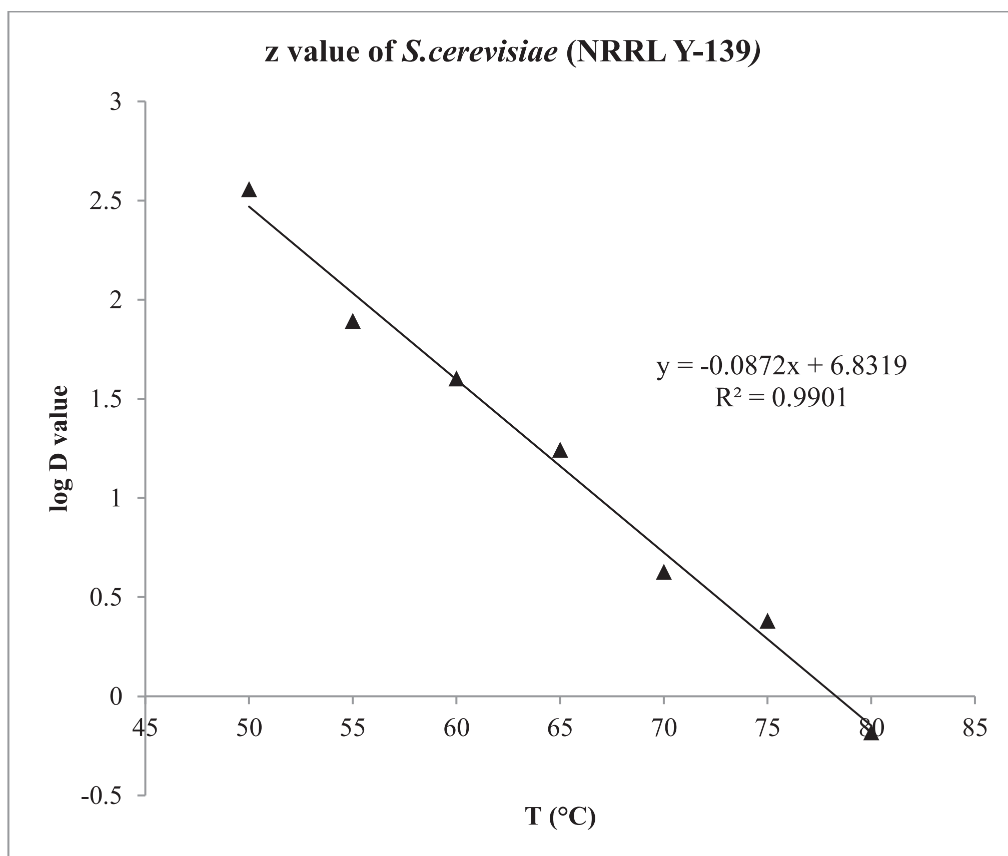


Figure A.4. Thermal Death Time (TDT) curve for *S. cerevisiae* (NRRL Y-139) inoculated into freshly squeezed verjuice

APPENDIX B

VERJUICE PASTEURIZATION
BY PULSED-UV LIGHT (PUV) TREATMENT

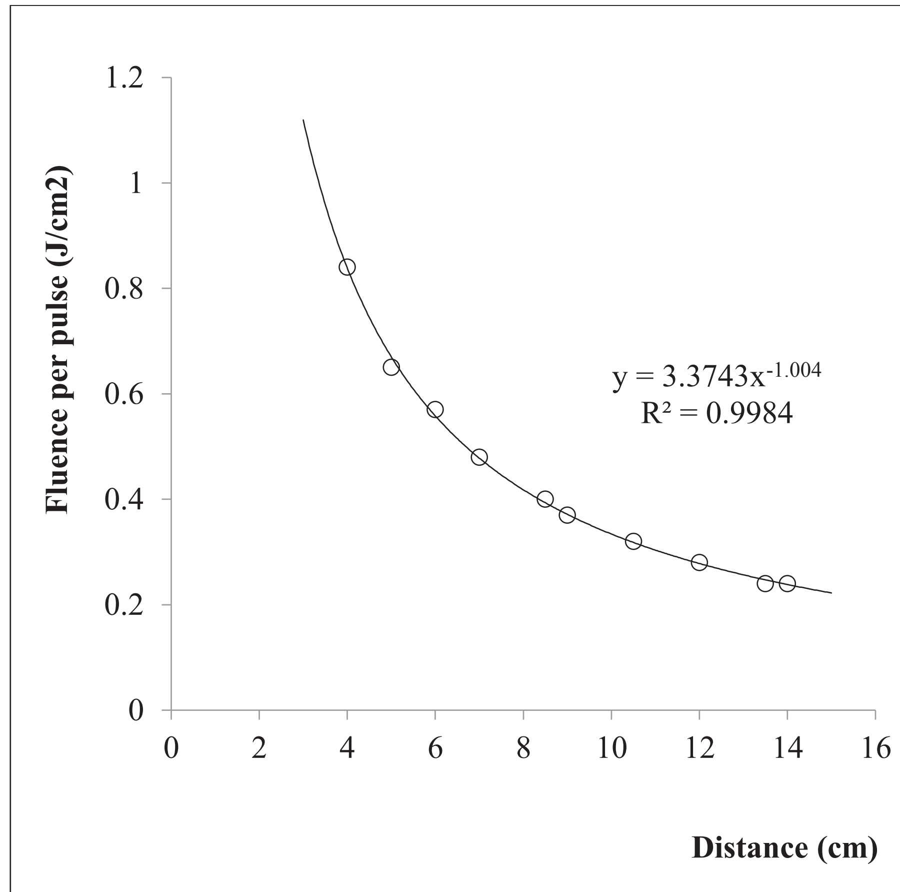


Figure B.1. The fluence of Pulse Light at different distances from the Xenon lamp in the Bench Top XeMaticA-2L system*. (*: the experiments were done at Lleida University before)

APPENDIX C

PRODUCTION OF VERJUICE POWDER

Table C.1. Onset (T_{onset}), glass transition (T_g) and endpoint temperatures ($T_{endpoint}$) of verjuice powders after freeze drying

	FD 48 h			**FD 72 h**		
	J+10%MD	**J+ 15%MD**	**J+20%MD**	**J+10%MD**	**J+ 15%MD**	**J+20%MD**
Tg_{onset}	9.26 ±0.25a	29.87 ±2.40bc	44.20 ±0.49c	24.07 ±11.59ab	41.18 ±0.30bc	44.95 ±0.81c
Tg	12.74 ±0.93a	43.88 ±0.96b	51.98 ±0.18b	36.41 ±9.71b	49.76 ±0.46b	51.54 ±1.85b
$Tg_{endpoint}$	30.15 ±1.94a	50.57 ±1.40bc	55.40 ±0.16c	41.95 ±7.46ab	53.23 ±0.83bc	55.30 ±1.36c

J: verjuice, MD: Maltodextrin, FD: freeze drying. Different small letters indicate differencies between samples based on One way ANOVA analyses compared by Tukey test ($p \leq 0.05$).

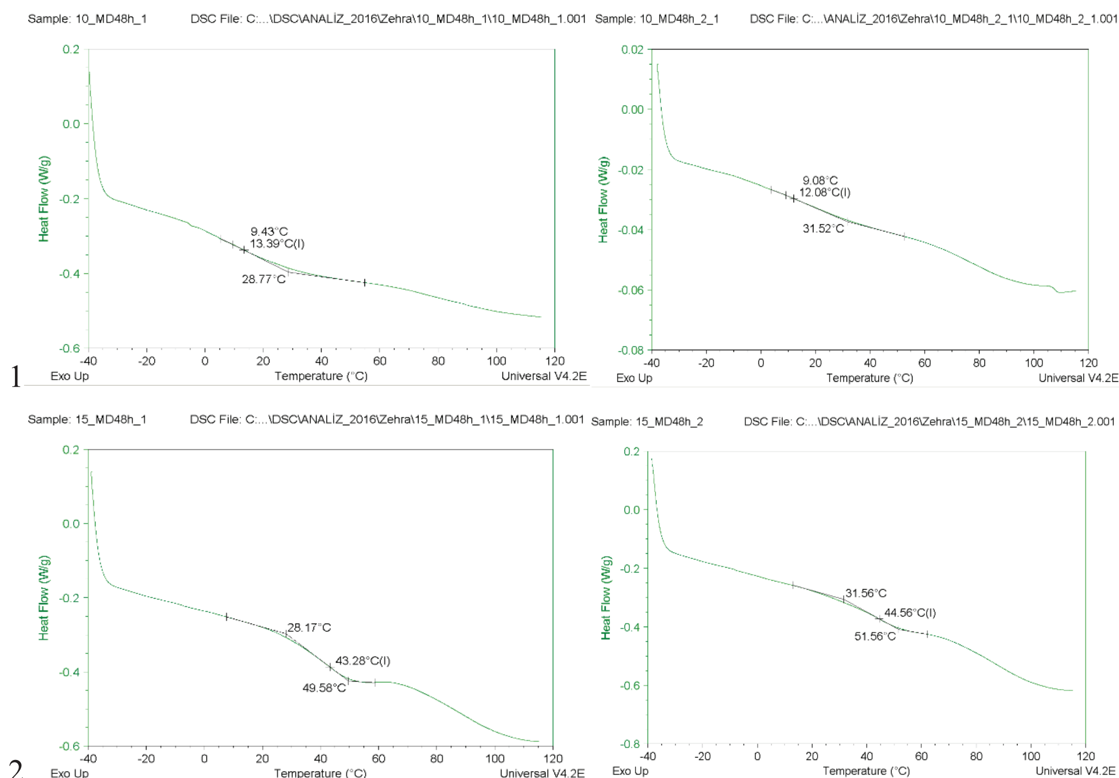


Figure C.1. Glass transition temperature diagrams of verjuice powders in Differential Scanning Colorimeter (DSC) analysis (1: J+10%MD_FD 48h, 2: J+15%MD_FD 48h 48h_1, 3: J+20%MD_FD 48h, 4: J+10%MD_FD 72h_2, 5: J+15%MD_FD 72h, 6: J+20%MD_FD 72h) (All numbered samples were shown as two graphs or two repeats)

(cont. on next page)

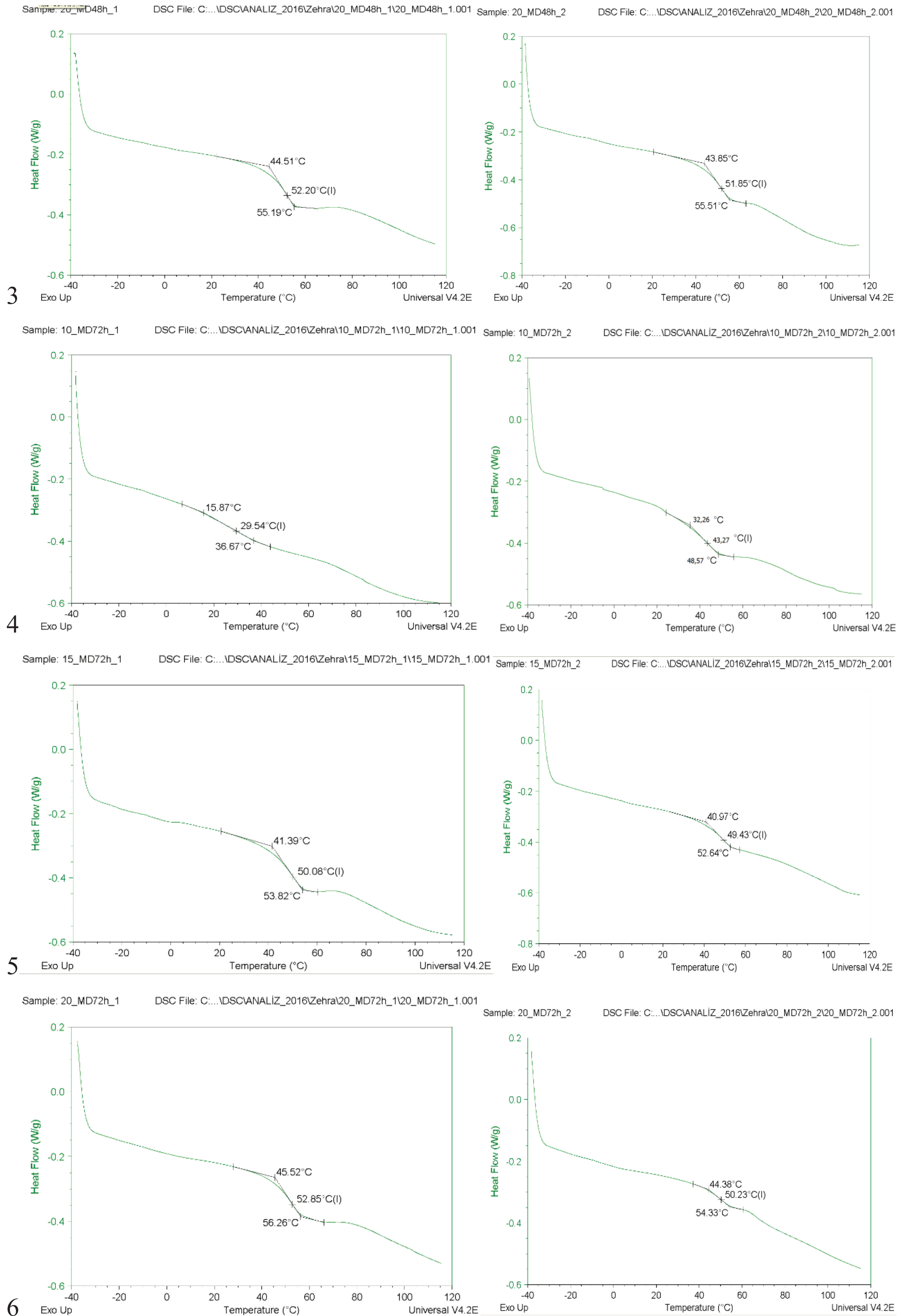


Figure C.1 (cont.)

APPENDIX D

PRODUCTION OF CONCENTRATED VERJUICE

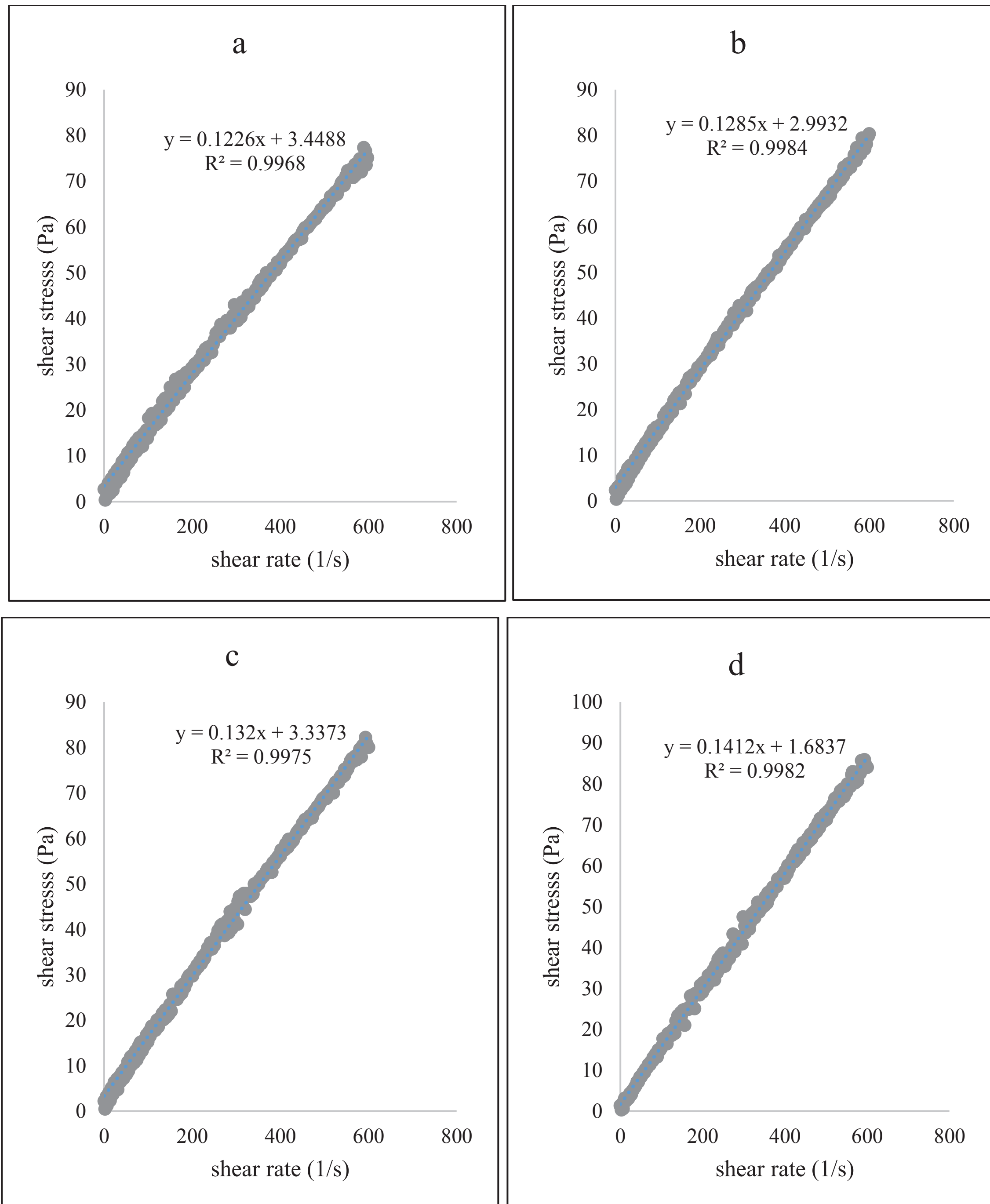


Figure D.1. Viscosity measurements* of concentrated verjuice produced by different vacuum evaporation conditions and traditional boiling (TB) (a: 60 °C/100 mbar, b: 70 °C/158 mbar, c: 80 °C/250 mbar, d: 100 °C/1013 mbar)

*: ramp test was performed by increasing (0-600 1/s) and decreasing (600-0 1/s) shear rate at 25 °C.

VITA

Zehra KAYA was born on October 12, 1986 in İzmir, Turkey. She graduated with a bachelor's degree in the Department of Food Engineering in Celal Bayar University, Manisa in 2007. She started the Master programme of the Department of Food Engineering in İzmir Institute of Technology in September of 2009, and received the Master of Science degree as a food engineer in February of 2012. She studied non-thermal UV-C inactivation of grape juice in her Master of Science period. The Food Engineering Department in İzmir Institute of Technology accepted her to study the Philosophy of Doctorate programme in 2013. Besides, she worked as a research assistant at Department of Food Engineering in İzmir Institute of Technology between 2013 and 2018. At the beginning of her Ph.D., her project related to the combined UV-C and mild heat pasteurization of verjuice was partially supported and awarded with the 3rd prize at the "Research & Development Project Market about Food" in 2012, and this was the starting point of her thesis. Currently, she has been working on the area of nonthermal processing, drying and concentration methods on fruit juices. In her Ph.D. education, she also worked as a visiting researcher with Dr. Olga Martin-Belloso and Dr. Robert Soliva-Fortuny at Food Technology Department in Lleida University, Spain for 6 months period (September 2016 and March 2017) to investigate the Pulsed UV Light technology in processing of fruit juices. During her Ph.D. period, she participated a lot of international congress, conferences, workshops and training, as well as presented many oral presentations and posters about her work area. Besides, she has two published and one under-review SCI articles about the nonthermal UV-C irradiation.