BIOACTIVE COMPOUND RETENTION AND SHELF LIFE EXTENSION OF STRAWBERRY JUICE BY SELECTED NONTHERMAL PROCESSING TECHNOLOGIES

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by
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ABSTRACT

BIOACTIVE COMPOUND RETENTION AND SHELF LIFE EXTENSION OF STRAWBERRY JUICE BY SELECTED NONTHERMAL PROCESSING TECHNOLOGIES

Shelf life extension of fruit juices while maintaining quality and functional properties is of great interest. Nonthermal food processing technologies have been emerged as innovative preservation methods alternative to thermal pasteurization of fruit juices.

The objectives of this Ph.D. thesis were to (i) ensure microbial safety of strawberry juice (SJ) by thermal and nonthermal technologies, i.e. high pressure processing (HPP), ultrasound (US), pulsed electric field (PEF) and UV-C irradiation; (ii) evaluate physicochemical and phytochemical properties of SJ after processing and during storage; (iii) extend microbial shelf life of SJ without unduly affecting quality. HPP (300 MPa, 1 min), US (120 μm, 5.15 J/mL, 55 °C, 3 min), PEF (35 kV/cm, 27 μs, 155 Hz), and thermal pasteurization (71.7 °C, 15 s) conditions were determined based on equivalent inactivation of *E.coli* (ATCC 11775) in SJ studied at Washington State University. UV-C irradiation (1.01 J/mL, 53.9 °C, 6 cycles, 5.8 min) and thermal pasteurization (72 °C, 101 s) conditions were determined considering inactivation of *E. coli* K-12 (ATCC 25253) in SJ studied at IZTECH.

Consequently, microbial shelf life of SJ treated by HPP, thermosonication, UVC+mild heating, thermal pasteurization was extended up to 42 days while PEF prolonged up to 35 days at refrigerated storage. Multivariate data analysis revealed that HPP and PEF enhanced phytochemical characteristics of SJ compared to thermosonication and thermal pasteurization. Although UV-C irradiation did not alter physcicochemical properties, total phenolic content, and antioxidant activities of SJ, total anthocyanin content was significantly decreased by UV-C light compared to untreated juice.

ÖZET

SEÇİLEN ISIL OLMAYAN İŞLEME TEKNOLOJİLERİ İLE ÇİLEK SUYUNUN BİYOAKTİF BİLEŞİKLERİNİN KORUNMASI VE RAF ÖMRÜNÜN ARTTIRILMASI

Meyve sularının kalite ve fonksiyonel özelliklerini koruyarak raf ömürlerinin uzatılması büyük önem taşımaktadır. Isıl olmayan gıda işleme teknolojileri meyve sularının termal pastörizasyonuna alternatif yenilikçi koruma yöntemleri olarak ortaya çıkmıştır.

Bu doktora çalışmasının amaçları (i) çilek suyunun termal pastörizasyon ve termal olmayan gıda işleme yöntemlerinden yüksek basınç (HPP), ultrases (US), darbeli elektrik alanı (PEF), ve UV-C ışınlama gibi teknolojiler ile mikrobiyal güvencesinin sağlanması, (ii) proses sonrasında ve depolama süresince çilek suyunun fizikokimyasal ve fitokimyasal özelliklerinin değerlendirilmesi, (iii) çilek suyunun kalitesini olumsuz bir şekilde etkilemeden mikrobiyal raf ömrünün arttırılmasıdır. HPP (300 MPa, 1 dk), US (120 μm, 5.15 J/mL, 55 °C, 3 dk), PEF (35kV/cm, 27 μs, 155 Hz), ve termal pastörizasyon (71.7 °C, 15 s) koşulları Washington State Üniversitesi'nde çalışılmış olup *E. coli* ATCC 11775 suşunun bahsi geçen teknolojiler tarafından eş değer inaktivasyonuna dayanarak belirlenmiştir. İzmir Yüksek Teknoloji Enstitüsü'nde çalışılan UV-C ışınlama (1.01 J/mL, 55 °C, 6 devir, 5.8 dk) ve termal pastörizasyon (72 °C, 101 s) koşulları *E. coli* K-12 (ATCC 25253) suşunun inaktivasyonuna dayanarak belirlenmiştir.

Sonuç olarak; yüksek basınç, termosonikasyon, ılımlı ısıl işlemle desteklenmiş UV-C ışınlama, termal pastörizasyon çilek suyunun mikrobiyal raf ömrünü 42 güne kadar uzatırken; darbeli elektrik alanı 35 güne kadar uzatmıştır. Çok değişkenli veri analizleri yüksek basınç ve elektrik alanı uygulamalarının termal pastörizasyon ve termosonikasyona kıyasla çilek sularının fitokimyasal özelliklerini geliştirdiğini göstermiştir. UV-C ışınlama ise çilek suyunun fizikokimyasal özelliklerini, toplam fenol içeriğini, ve antioksidan aktivitesini önemli ölçüde etkilememesine rağmen, toplam antosiyanin içeriğini işlem görmemiş çilek suyuna kıyasla önemli ölçüde azaltmıştır.

This PhD thesis is dedicated to my dear parents, Naima Yıldız and Gürbüz Yıldız, my brother Haluk Yıldız, and my sister Ülkü Selin Yıldız for making all of my achievements possible; and to the people who have made me who I am today.

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LIST OF SYMBOLS AND ABBREVIATIONS

FDA	Food and Drug Administration
HPP	High pressure processing
US	Ultrasonication
PEF	Pulsed electric field
SJ	Strawberry juice
MS	Model solution
AC	Acoustic cavitation
τ	Shear stress
γ	Shear rate
I_0	Incident intensity (mW/cm^2)
I_{ave}	Average intensity (mW/cm^2)
t	Treatment time
$[c_{triiodide}]$	Initial concentration of iodide (M) formed by photoreaction
N_0	Initial microbial population
N	Survival microbial population at any treatment time
k	Inactivation rate constant (min^{-1}) for log-linear model
α	Scale parameter in Weibull model
β	Shape parameter in Weibull model
TSA	Tryptic soy agar
PDA	Potato dextrose agar
PCA	Plate count agar (for microbiology)
VRBA	Violet red bile agar
CFU	Colony forming unit
RMSE	Root mean square error
μ	Apparent viscosity (kg/m.s)
L*	Lightness-darkness
a*	Redness-greenness
b*	Yellowness-blueness
ΔE	Total color difference
BI	Browning index
TA	Titratable acidity

f	Normality factor
E	Miliequivalent weight of citric acid
TPC	Total phenolic content
TAC	Total anthocyanin content
RSA	Radical scavenging activity
EFI	Electrical field intensity
MW	Molecular weight (g/mol)
df	Dilution factor
L	Path length of spectrophotometer cuvette (cm)
ε	Molar extinction coefficient (L/mol.cm)
PCA	Principal component analysis (for data analysis)
HCA	Hierarchical cluster analysis

CHAPTER 1

INTRODUCTION

Fruit juices are widely consumed food products in all around the world. Assuring the safety and quality of fruit juices until the product reaches to the consumer is a major concern. Depending on the nutritional composition and type of fruit, the juices are susceptible to spoilage by *Lactobacillus* and *Leuconostoc* (Winniczuk & Parish, 1997), *Acetobacter aceti* and *Acetobacter pasteurianus* (Worobo & Splittstoesser, 2005), *Candida, Pichia, Rhodotorula, Torulopsis, Saccharomyces, Zysossaccharomyces, Hansenula,* and *Trichosporon* genera (Renard, di Marco, Egea-Cortines, & Weiss, 2008). Moreover, several foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella* spp and *Cryptosporidium parvum* (CDC, 1996, 1999, 2007) have been associated with some outbreaks and foodborne illnesses. Therefore, more stringent strategies have been developed in order to control the microbiological quality and safety of fruit juices (Tribst, Sant'Ana, & de Massaguer, 2009). The regulation proposed by Food and Drug Administration (FDA) requires a 5-log reduction in the most pertinent microorganism by processing (FDA, 1998). The 5-log reduction performance standard is well satisfied by thermal pasteurization which has been widely applied as a conventional method for the preservation of fruit juices. However, thermal processing has undesirable effects such as loss of nutrients, degradation of bioactive compounds, and alteration of sensorial attributes in the final product. Although the microbial safety is the first concern to be satisfied; preservation or improvement of desirable sensorial attributes, nutritional and health related properties of fruit juices should also be taken into account in order to meet consumers' demands. Nowadays, consumers have an increasing demand for fresh-like foods with high quality, microbiologically safe, as well as rich in terms of natural flavor, taste, nutrients, and bioactive compounds (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007; Sanchez-Moreno, De Ancos, Plaza, Elez-Martinez, & Cano, 2009). Therefore, scientists have showed interest in exploring innovative food processing technologies. Alternative nonthermal technologies have potential to replace the traditional processing

methods with minor changes in fresh-like properties or increased content in health-related phytochemicals.

In this respect, nonthermal processing technologies including high pressure processing (HPP), ultrasonication (US), pulsed electric fields (PEF), UV-C irradiation have gained special attention as alternative methods to thermal processing to obtain microbiologically safe food with high nutritional and functional quality, and a longer shelf-life. HPP is one of the novel technologies allowing processing of the food at elevated pressure with or without addition of heating. The desired microbial inactivation levels as well as food attributes can be achieved by HPP (Ramaswamy, Balasubramaniam, & Kaletunç, 2011). US technology has been gaining attention in food processing due to its potential to satisfy the pasteurization requirements. The effectiveness of this technology arises from the cavitation generated by sound waves passing through the medium. Cavitation enables the microbial inactivation by disrupting the cell membrane (Bermúdez-Aguirre & Barbosa Cánovas, 2011). PEF is a nonthermal food processing technology that leads inactivation of microorganisms with minimal change in food quality by subjecting liquid or semi-liquid food products to high voltage pulses of electricity (Ramaswamy, Jin, Balasubramaniam, & Zhang, 2011). UV-C light, on the other hand, has gained interest in the food industry as a method to be used for decontamination of surfaces, sterilization of air, and disinfection of liquids (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). In addition; hurdle approach, which employs a combination of different processing technologies, has been gaining interest in preservation of food due to the potential influence on increasing stability, safety and quality of the products.

The objectives of this Ph.D. thesis were (i) to investigate the efficacy of selected nonthermal processes such as HPP, US, PEF and UV-C light for the microbial safety of strawberry juice (SJ), (ii) to determine effects of each process on physicochemical and optical properties, as well as health related bioactive compounds, (iii) to extend the shelf life of treated juices. The selected nonthermal technologies were compared with the untreated and thermally pasteurized juice samples.

Chapter 2 provides theoretical background about fruit juices in terms of microbial and chemical aspects by putting special emphasis on berry products. Microbial safety and quality of juice products were discussed by considering spoilage and pathogenic microorganisms. Then, the major bioactive compounds present in fruit juices and their benefits were summarized. Preservation of fruit juices by thermal and

nonthermal processing technologies as well as hurdle technology was outlined in this chapter.

Each technology has several processing parameters and different mechanism of action on the inactivation of microorganism. Therefore, Chapter 3 covers the fundamental principles, microbial inactivation mechanism, equipment and processing parameters, advantages and disadvantages of each nonthermal processing technology that has been studied in this PhD thesis. Then, the applications of high pressure, pulsed electric field, ultrasound, and UV-C light treatments were reviewed considering microbiological quality, physicochemical and phytochemical properties of fruit juices.

In Chapter 4, an overview of methodologies used both at Washington State University and Izmir Institute of Technology were given including preparation of strawberry juice, physicochemical assays, analytical assays and data analysis. Additional methodologies related to each technology were detailed in corresponding chapters if necessary.

In Chapter 5, processing conditions of strawberry juice by HPP, PEF and US were determined considering inoculation of a microorganism of concern (*i.e.* a surrogate of *E. coli* O157:H7) based on the equivalent processing approach. Equivalent processing is a concept of a fair comparison of different processing technologies on the basis of joint accomplishment. For this purpose, the juice inoculated with acid adapted *E. coli* (ATCC 11755) was subjected to by HPP, US and PEF processes. With respect to HPP, the inactivation kinetics of target microorganism was monitored at varying pressure and time. US process was combined with mild heating at different temperatures. A central composite design was constructed for PEF considering electrical field intensity and frequency as independent variables at a constant pulse width and flow rate. In order to satisfy FDA's 5-log reduction requirement in the pertinent microorganism, the processing conditions of each technology that accomplished at least 5-log reduction were selected for the pasteurization of strawberry juice. Results were validated by applying the same conditions to strawberry juice having natural flora. The total mesophilic aerobic count and yeast-mold count were determined in processed juice. This part of thesis was carried out at Washington State University, Pullman, WA, USA.

In Chapter 6, the processing conditions determined in Chapter 5 were applied for pasteurization of the freshly squeezed strawberry juice. Then, the microbiological quality, physicochemical and phytochemical properties of strawberry juice before and

after treatments were evaluated. Regarding the microbiological quality, total mesophilic aerobic plate and yeast-mold counts were monitored. pH, titratable acidity, and total soluble content were measured as physicochemical attributes of SJ. Phytochemical properties were analyzed in terms of total phenolic content, antioxidant capacity and total anthocyanin content. This part of thesis was carried out at Washington State University, Pullman, WA, USA.

Chapter 7 covers the shelf life study of SJ treated by high pressure, US, PEF and conventional thermal pasteurization. The freshly squeezed SJ samples treated by HPP, PEF, US and conventional thermal pasteurization were stored at refrigerated conditions for 42 days. The microbial quality, physicochemical, and phytochemical properties of untreated and treated SJ samples were monitored during storage period. This part of thesis was also carried out at Washington State University, Pullman, WA, USA.

In Chapter 8, the processing conditions of SJ by UV-C light were studied by inoculating *E.coli* K-12, as a surrogate of *E.coli* O157: H7, into SJ. Different UV-C lamp configurations, flow rates, temperatures, thereby different UV-C intensities, were tested in order to find the conditions that accomplished at least 5-log reductions in the number of target microorganism. The combination of UV-C light with mild heating was also investigated along with the conventional method.

Chapter 9 covers the shelf life study of freshly squeezed SJ processed under the conditions that were previously determined in Chapter 8. For the preservation of strawberry juice, UV-C assisted with mild heating and conventional thermal pasteurization were applied; and the processed juices were compared in terms of microbial quality, physicochemical and phytochemical properties. During 42 days of refrigerated storage, microbial stability and physicochemical attributes of SJ were monitored.

Chapter 10 concludes the thesis. A summary of the main findings is presented. It also contains recommendations for future research and the closing remarks.

CHAPTER 2

FRUIT JUICE AND PRESERVATION METHODS

2.1. Introduction

Fruit juice is the unfermented but fermentable liquid obtained from the mature and fresh fruits. The juice is prepared by suitable processes, which maintain the essential physical, chemical, organoleptic and nutritional characteristics of the juices of the fruit from which it comes. The juice may be cloudy or clear with aromatic substances and volatile flavor components (Codex, 2005). Juicing fruit has become a convenient alternative for some people, allowing them to quickly drink the needed daily requirements. The nutritional quality of fruits and fruit juices are determined based on their macronutrients (proteins, carbohydrates, and lipids) and micronutrients (vitamins and minerals, fatty acids, and essential aminoacids) contents. Besides; bioactive compounds, *i.e.* phytochemicals, increase functionality and health promoting properties of fruit and fruit juice. These compounds in fruits and juices work in collaboration with nutrients and dietary fiber to protect human body against many diseases, including cancer, high blood pressure, heart disease, and cataracts (Sanchez-Moreno, De Ancos, Plaza, Elez-Martinez, & Cano, 2009). Due to their health promoting components, fruit and fruit juices have considered to be an important part of a human diet.

In this chapter, bioactive compounds in fruits and their health benefits, the constituents of strawberry and strawberry juice, microbial safey and quality of fruit juices, and fruit juice preservation methods including thermal and nonthermal processing technologies as well as hurdle processing approach were covered.

2.2. Bioactive Compounds and Health Benefits of Fruits

Free radicals are the reactive chemical species with a single unpaired electron in an outer orbit (Riley, 1994). The energy created due to this unstable configuration tends to release through several reactions with proteins, lipids, carbohydrates, and nucleic

acids. The free radicals that can damage biological systems are oxygen-free radicals. In general, they are called as “reactive oxygen species” (ROS) (Rahman, 2007). ROS act as a causative factor in many diseases such as cardiovascular diseases, cancer, diabetes mellitus, ageing and neurodegenerative diseases (Azizova, 2002). Superoxide radical, hydrogen peroxide, hydroxyl free radical, singlet oxygen and nitric oxide are the most common forms of ROS with biological activities including DNA mutation, alteration of gene expression, cell apoptosis, lipid peroxidation and protein degradation (Nordberg & Arner, 2001).

Consumption of fruits and vegetables has been inversely associated with the risk of chronic degenerative diseases (Leifert & Abeywardena, 2008). The natural antioxidants (phytochemicals) in fruits are considered to have health promoting properties due to their high antioxidant ability and free radical scavenging capacity (Kahkonen, Hopia, & Heinonen, 2001). The principle function of antioxidants is based on delaying the oxidation of other molecules by inhibiting the initiation or propagation of ROS related oxidation reactions (Namiki, 1990).

Phytochemicals are non-nutritive, but biologically active plant metabolites which have functions such as pigmentation, antioxidation, protection against UV-light in plants (Fennema, 1996). Phenolic compounds are the largest category of phytochemicals and the most widely distributed in the plant kingdom. They are plant secondary metabolites, which are generally synthesized through the shikimic acid pathway (Dixon & Paiva, 1995; Vasco, 2009). A benzene ring or another complex aromatic ring structures are attached with one or more hydroxyl groups (Tokuşoğlu, Swanson, & Barbosa-Cánovas, 2014). Phenolic compounds can be basically classified as phenolic acids (hydroxycinnamic and hydroxybenzoic acid), flavonoids (anthocyanins, flavonols, proanthocyanidins, flavonols, flavones, flavanones, isoflavones), tannins, and lignans (Fennema, 1996). Figure 2.1 shows the structure of some phenolic compounds (Dai & Mumper, 2010).

Phenolic compounds play some important roles in fruits in terms of visual appearance, taste and aroma. For example, these compounds are responsible for most of the blue, purple, red and intermediate colors of fruits (Fennema, 1996). They also have significant functions in biological systems including radical scavenging activity, and protecting proteins, lipids, and DNA from oxidative damage. As a result phenolic compounds in fruit and fruit juices are considered as biologically active compounds that may possess some disease-preventive properties. Therefore, consumption of fruit and

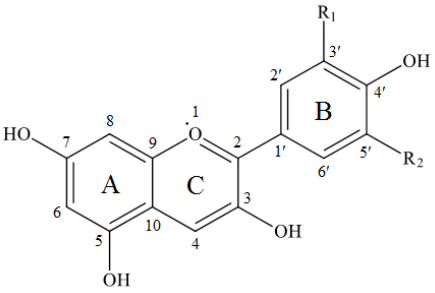
fruit juices has become a dietary concern worldwide since they are rich sources of bioactive compounds or phytochemicals. The major bioactive antioxidant compounds of fruit and vegetables juices are vitamin C and phenolic compounds as well as carotenoids (Rodriguez-Roque, Rojas-Grau, Elez-Martinez, & Martin-Belloso, 2013).

Anthocyanins are a widespread group of plant phenolic compounds which are responsible from red and blue pigments of the plants, flowers, and fruits leading to attraction processes related to pollination and seed dissemination (Cheynier, 2012). They are water soluble pigments found in the external layers of the skin as well as in vacuoles in the cell. Anthocyanins are composed by anthocyanidins and their glucosides (Shahidi & Naczk, 2004). They have been regarded as a natural alternative to replace synthetic food colorants. Recently, increased attention has been given to their potential health benefits in preventing heart diseases and cancers due to their powerful antioxidant properties (Hannum, 2004).

Flavonols play protective role in plant tissues against UV irradiation. Quercetin and kaempferol are the best known flavonols (Cheynier, 2012). Tannins, on the other hand, are divided into two categories as hydrolysable and condensed non-hydrolysable (proanthocyanidins) tannins. Derivatives of gallic acid and ellagic acid, (-)epicatechin, and (+)catechin are the examples of tannins in fruits. Tannins are responsible for the taste and color of fruits. They can stabilize anthocyanins by binding them for the formation of copolymers (Szajdek & Borowska, 2008).

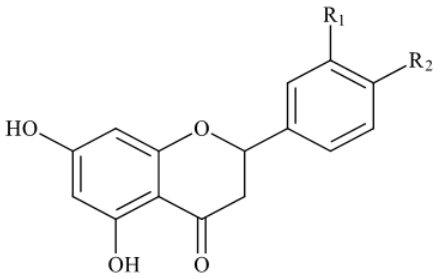
Phenolic acids in fruits present as cinnamic acid benzoic acid derivatives. p-hydroxybenzoic acid, salicylic acid, gallic acid and ellagic acid are the main examples of hydroxybenzoic acids while p-coumaric acid, caffeic acid and ferulic acid are classified in the group of cinnamic acid derivatives. Chlorogenic acid, which is the ester form of caffeic acid and quinic acid, has been also found in high quantities in fruits (Szajdek & Borowska, 2008).

Anthocyanidin



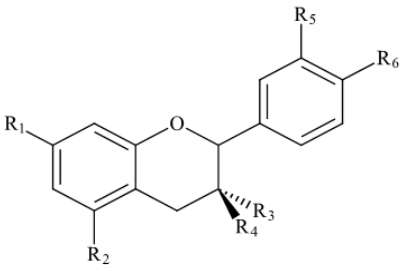
Delphinidin: $R_1 = R_2 = OH$
Cyanidin: $R_1 = OH$, $R_2 = H$

Flavanone



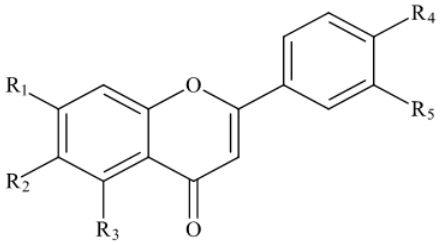
Hesperetin: $R_1 = OH$, $R_2 = OCH_3$
Naringenin: $R_1 = H$, $R_2 = OH$

Flavanol



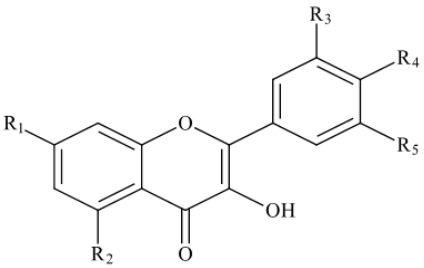
Catechin: $R_1 = R_2 = R_4 = R_5 = R_6 = OH$, $R_3 = H$
Epicatechin: $R_1 = R_2 = R_3 = R_5 = R_6 = OH$, $R_4 = H$

Flavone



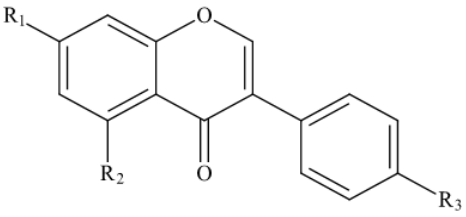
Apigenin: $R_1 = R_3 = R_4 = OH$, $R_2 = R_5 = H$
Luteonin: $R_1 = R_3 = R_4 = R_5 = OH$, $R_2 = H$

Flavonol



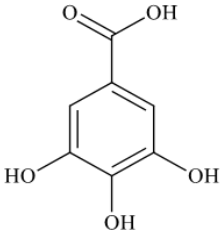
Quercetin: $R_1 = R_2 = R_3 = R_4 = OH$, $R_5 = H$
Myricetin: $R_1 = R_2 = R_3 = R_4 = R_5 = OH$

Isoflavone



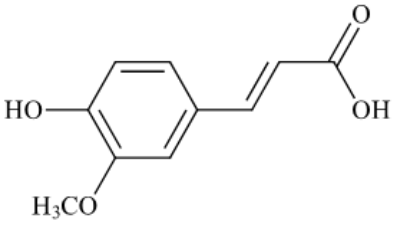
Genistein: $R_1 = R_2 = R_3 = OH$
Daidzein: $R_1 = R_3 = OH$, $R_2 = H$

Hydroxybenzoic acid



Gallic acid

Hydroxycinnamic acid



Ferulic acid

Figure 2.1. Structures of flavonoids and phenolic acids
(Source: Dai and Mumper, 2010)

2.3. Strawberry and Strawberry Juice (SJ)

Berry fruits have been reported as one of the rich source of phenolic compounds such as phenolic acids, flavonoids, stilbenes, and tannins. In recent years many studies have been focused on bioactive compounds in berries, their characterization and utilization in functional foods due the health promoting properties. The concentration of phenolics in berries varies depending on the species, variety, geographic region, ripeness, climate, and storage conditions (Paredes-Lopez, Cervantes-Ceja, Vigna-Perez, & Hernandez-Perez, 2010).

Strawberries (*Fragaria* x *ananassa*) are commonly consumed fruits due to the content of essential nutrients and bioactive phytochemicals. Table A.1 in Appendix A shows the nutritional composition of strawberries. The dietary fiber (2 g/100g) and fructose (2.44 g/100g) contents may regulate the blood sugar levels by slowing the digestion (Giampieri et al., 2012). Vitamin C, on the other hand, is another important constituent in the nutrient profile of strawberry. It is one of the important water-soluble vitamins. The vitamin C content of strawberry has been varied from 23.8 to 84.7 mg per 10 g of fresh weight depending on species, variety, cultivation and climate conditions, ripeness and storage time (Szajdek & Borowska, 2008). The amount of Vitamin C in strawberry has been reported to be 58.8 mg/100 g (Table A.1).

In addition to nutritional constituents, flavonoids and other phenolic compounds (phenolic acids, stilbenes, lignans), are mostly studied compounds due to their potential health-related properties (Sanchez-Moreno et al., 2009). The health benefits of strawberry phenolics are attributed to their antioxidant and anti-inflammatory, direct and indirect antimicrobial, anti-allergy, and anti-hypertensive properties, as well as the capacity for inhibiting the activities of some physiological enzymes and receptors, preventing oxidative stress-related diseases (Wang, Cao, & Prior, 1996). Giampieri et al. (2015) thoroughly reviewed the relation between strawberry and health. Strawberry phenolics can promote human health since they are able to (i) block and detoxify free radicals, (ii) regulate gene expression involving in metabolism and antioxidant defense mechanism, (iii) avoid DNA damage (Giampieri et al., 2015).

The important class of strawberry polyphenols are flavonoids, mainly anthocyanins (pelargonidin and cyanidin derivatives), ellagitannins, flavonols (catechins and procyanidins), and phenolic acids (Giampieri et al., 2015). However, the

polyphenolic composition of strawberry fruits depends significantly on the genotype (Anttonen, Hoppula, Nestby, Verheul, & Karjalainen, 2006; Capocasa, Scalzo, Mezzetti, & Battino, 2008), as well as agricultural factors, environment, and maturity (Aaby, Mazur, Nes, & Skrede, 2012; Pineli et al., 2011; Tulipani et al., 2008). The total phenolic content of strawberry fruit has been reported as 317.2-443.4 mg per 100 g of fresh weight (Zheng, Wang, Wang, & Zheng, 2007). Table A.2 in Appendix A represents the flavonoid composition of strawberries. Anthocyanins are the commonly known phenolic compounds in strawberries (Aaby, Skrede, & Wrolstad, 2005; Clifford, 2000). Regardless of genetic and environmental factors, pelargonidin-3-glucoside is the major anthocyanin in strawberries (da Silva, Escribano-Bailon, Alonso, Rivas-Gonzalo, & Santos-Buelga, 2007). Aaby et al. (2012) has characterized twenty seven strawberry cultivars in terms of anthocyanins, flavan-3-ols, and ellagitannins. It has been reported that the concentration of anthocyanins varied from 8.5 to 65.9 mg/100 g of fresh weight among cultivars (Aaby et al., 2012). The major anthocyanins in strawberry are pelargonidin 3-glucoside, cyanidin 3-glucoside, and pelargonidin 3-rutinoside (da Silva et al., 2007). Among different cultivars, the concentration of flavan-3-ols has been detected inbetween 11-45 mg/100 g of fw; while the ellagitannins have varied from 7.7 to 18.2 mg/100 g of fw (Aaby et al., 2012).

The production quantities of strawberry for each country have been reported by Food and Agriculture Organization of the United Nations (FAO). Figure 2.1 shows the percentage of production quantity of several countries in 2013 based on the statistical data obtained from FAO (FAOSTAT, 2016). The major leading countries for strawberry production have been recorded as China (39 %), United States (18 %), Mexico (5 %), Turkey (5 %), and Spain (4 %). The production quantity of strawberry in Turkey has been compiled by Turkish Statistical Institute (TSI) since 1988 as illustrated in Figure 2.2. The yield per year shows a remarkable increase over years. Considering the last 10 years, the production yield of strawberry in Turkey has been increased from 200.000 tons to 375.800 tons (TSI, 2016). From commercial and economical point of view, strawberries are consumed either fresh or in processed form. The processed forms of strawberries include juices, jams, or jellies (Giampieri et al., 2012). Besides, beverages and yogurts supplemented with strawberry are also alternative commercial products in the market.

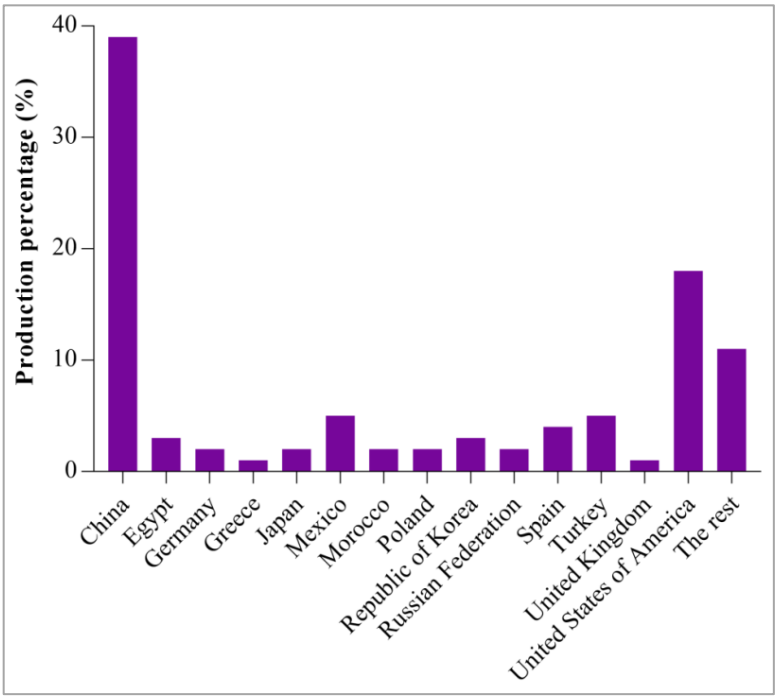


Figure 2.2. Production of strawberry in the world in 2013
(Source: FAOSTAT, 2016)

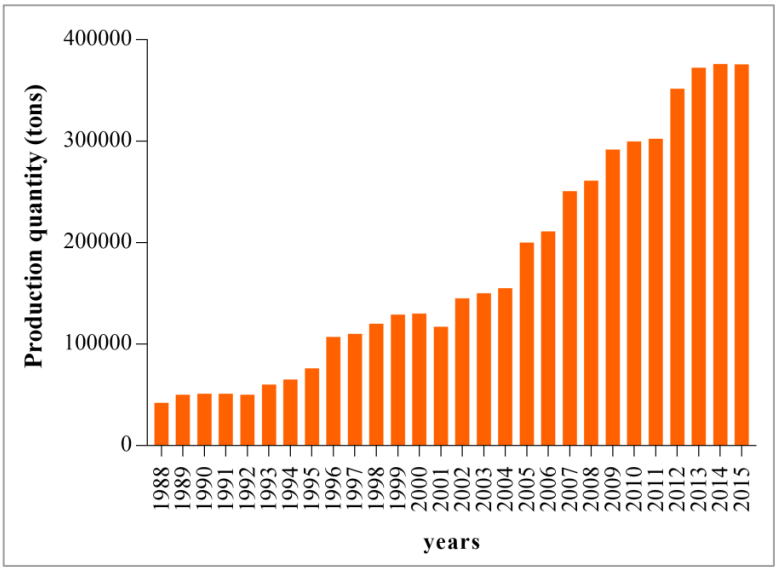


Figure 2.3. Production of strawberry in Turkey since 1988
(Source: TSI, 2016)

Among different types of products, utilization of fresh fruits as juice has many practical advantages (Bates, Morris, & Crandall, 2001):

- Soft fruits tend to deteriorate before, during or after harvesting since they cannot keep the intact form over long periods
- It is more convenient to consume juices compared to whole fruit due to easy to handle containers.
- Very young or elderly people may have problems for peeling or eating the fresh fruit. Thus, consumption of juices is an easy and effective way since they can be an alternative to benefit from the nutrients.
- The juices can be blended with another nutrient or phytochemicals in order to be acted as a carrier for these compounds.
- Processing efficacy, safety, and quality parameters are easier to standardize for fluid foods including juices compared to solid foods.
- New formulations can be developed by blending the juices which is not naturally available.
- The juices, which are rich in flavor, can be used for the production of some co-products such as syrups, ice cream, smoothies, bakery ingredients, as well as flavoring agent.
- Considering modern processing, packaging and distribution systems, juicing allows consumers to have safe, stable, and appealing product which has convenience and accessibility regardless of season.

These advantages are also valid for strawberry and strawberry juice. According to the database of Turkish Fruit Juice Industry Association (MEYED), the amount of strawberry used for juice production in Turkey was 4.100 and 7.700 tons in 2007 and 2008, respectively. Even though the major types of fruits for juice production are apple, peach, apricot, and cherry; the strawberry can be used either in juice blends or concentrates in order to increase the product range (MEYED, 2017). However, processing strawberries into different forms of food products may cause loss of attractive color which is provided by pelargonidin-3-glucoside as the responsible anthocyanin for the bright and appealing color of strawberries (Garzon & Wrolstad, 2002). The nutritional quality of strawberry fruit may also change when converted to juice form. Klopotek et al. (2005) processed strawberry into different types of products

such as juice, nectar, wine, and puree in order to evaluate the changes in health related constituents. The authors stated that the content of ascorbic acid, total phenolics, and antioxidant capacity decreased when the strawberry was processed to juice. The reason for the loss in such properties was attributed to the use of high temperature and pre-steps during processing fresh strawberry to juice. While the ascorbic acid content of strawberry was 104 %, it reduced to 36 % in pasteurized juice. Similarly, total phenolics were higher in fruit (257 %) compared to the pasteurized juice (35.6 %). Total anthocyanins, on the other hand, were 42 % and 67 % for strawberry and pasteurized strawberry juice, respectively. Even though total anthocyanin content was slightly increased in the pasteurized juice, this is believed to be resulted from mashing and pressing effect. Total anthocyanin content was increased from 42 % to 101 % during mashing and pressing operations. On the other hand, the thermal pasteurization actually caused the loss of total anthocyanins because their level reduced to 67 % after thermal processing (Klopotek, Otto, & Bohm, 2005). In order to avoid the detrimental effect of thermal processing and preserve the anthocyanins and some other quality parameters, the strawberry juice has been processed by alternative technologies such as high intensity PEF (Aguilo-Aguayo, Oms-Oliu, Soliva-Fortuny, & Martin-Belloso, 2009; Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2009), ultrasonication (Bhat & Goh, 2017; Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009a; Tiwari, O'Donnell, Patras, & Cullen, 2008), ozone processing (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009b); addition of chemical preservatives at refrigeration conditions (Ayub, Ullah, Muhammad, & Zeb, 2010), HPP (Cao, Liu, Wu, Liao, & Hu, 2014; Rodrigo, van Loey, & Hendrickx, 2007); and pulsed light (Ferrario, Alzamora, & Guerrero, 2015).

In this study, strawberry juice was selected as a model liquid food based on its health promoting properties aroused from bioactive constituents of strawberry fruit as well as prevalence of strawberry in the world. In this regard, this PhD thesis provides a comparative study for the evaluation of alternative nonthermal pasteurization technologies to preserve the microbial and nutritional quality of strawberry juice using equivalent processing approach.

2.4. Fruit Juice Preservation Methods

2.4.1. Microbial Safety and Quality of Fruit Juice

Fruit juice is a low acidic beverage. It is susceptible to spoilage by moulds, yeasts and aciduric bacteria such as *Lactobacillus*, *Leuconostoc* and *Acetobacter* spp. which lead to off-flavor, odor and color deterioration. The spoilage depends on the type of microorganism and variety of the juice (Ray, 2004; Tournas, Heeres, & Burgess, 2006). There are several kinds of microorganisms that can survive in fruit juices and cause spoilage. Some aerobes such as *Bacillus coagulans* and *Bacillus megaterium* cause flat-sour type spoilage. On the other hand, some anaerobic spore-forming bacteria such as *Clostridium butyricum* and *Clostridium pasteurianum* produce gas and gas and butyric odors (Silva & Gibbs, 2004). Yeasts and molds, *Lactobacillus*, *Leuconostoc* and thermophilic *Bacillus* are the microorganisms that can cause problems in fruit juice (Tran & Farid, 2004). Moreover, *Salmonella* and *Escherichia coli* O157:H7 outbreaks indicated that fruit juices are the potential sources for pathogenic microorganisms (Cook et al., 1998). The spoilage of apple juice or apple cider has been related to *Saccharomyces cerevisiae* (Basaran, Quintero-Ramos, Moake, Churey, & Worobo, 2004; Guerrero-Beltran & Barbosa-Canovas, 2004). Several cases of *Salmonella* outbreaks have been associated with the consumption of orange, grapefruit, watermelon, mamey, and lemonade juice. Many other cases related to foodborne illness outbreak caused by *E. coli* O157:H7 has been linked to consumption of apple cider, apple juice, and orange juice as shown in Table 2.1 (Danyluk, Goodrich-Schneider, Schneider, Harris, & Worobo, 2012; Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martin-Belloso, 2009).

Consequently, a regulation proposed by Food and Drug Administration (FDA) requires a 5-log reduction in the most pertinent microorganism that must be attained by processing (FDA, 2001). Therefore, the spoilage and contamination of a fruit juice are the major concerns and microorganisms must be prevented in the juice by applying several preservation methods in the processing (Bates et al., 2001; Ray, 2004; Tournas et al., 2006).

Table 2.1. Outbreaks of human foodborne disease due to various microorganisms associated with juice

Adopted from	Product	Causative factor	Year	Cases
Raybaudi-Massilia et al. (2009)				
	Orange juice (unpasteurized)	*Salmonella* ser. Typhimurium	2005	157
	Orange, grapefruit, and lemonade juice	*Salmonella* ser. Enteritidis	2000	74
	Orange juice (unpasteurized)	*Salmonella* ser. Muenchen	1999	398
	Orange juice	*Salmonella* ser. Typhimurium	1999	427
	Orange juice (unpasteurized)	*Salmonella* ser. Anatum	1999	10
	Orange juice	*Salmonella* ser. Hartford, and Gaminara	1995	62
	Orange juice	*E. coli* O157:H7	1992	6
	Orange juice	*Salmonella* ser. Enteritidis	1991	600
	Mamey juice (unpasteurized)	*Salmonella* ser. Typhimurium	1999	13
	Apple cider (unpasteurized)	*E. coli* O157:H7	1999	5
	Apple cider (unpasteurized)	*E. coli* O157:H7	1997	6
	Apple cider (unpasteurized)	*E. coli* O157:H7	1996	56
	Apple cider (unpasteurized)	*E. coli* O157:H7	1996	14
	Apple cider (unpasteurized)	*E. coli* O157:H7	1996	6
	Apple cider	*E. coli* O157:H7	1991	23
	Apple cider	*Salmonella* ser. Typhimurium	1974	296
	Apple juice	*E. coli* O157:H7	1998	14
	Apple juice (unpasteurized)	*E. coli* O157:H7	1996	71
	Apple juice (unpasteurized)	*E. coli* O157:H7	1980	14
	Watermelon juice	*Salmonella* ser. Javiana	1991	39
Danyluck et al. (2012)				
	Apple juice (unpasteurized)	*E. coli* O157:H7	2010	7
	Apple juice (unpasteurized)	*E. coli* O157:H7	2008	7
	Apple juice (unpasteurized)	*E. coli* O157:H7	2007	9
	Apple juice (unpasteurized)	*E. coli* O157:H7	2005	4
	Apple juice (unpasteurized)	*E. coli* O111 and *C. parvum*	2004	212
	Apple juice (unpasteurized)	*C. parvum*	2003	144
	Carrot juice (pasteurized)	*C. botulinum*	2006	4

2.4.2. Preservation Methods

Fruit juices are very important products in the global market offering extensive possibilities for new value added products to meet consumer demand for accessibility, nutrition, and health (Aneja, Dhiman, Aggarwal, & Aneja, 2014). Foodborne disease outbreaks and spoilage problems are the major concern to fruit juice industry worldwide.

Thermal processing is the most commonly used method for fruit juice pasteurization. The concept of pasteurization has been traditionally defined on the basis of time-temperature process. According to this definition, pasteurization is a process that applies heat under 100°C to destroy vegetative pathogen and spoilage microorganism in foods which has pH value under 4.5 (Fellows, 2009).

However, the conventional preservation methods by heat treatment may cause loss of nutrients, quality and fresh-like properties of the product. Therefore, innovative nonthermal technologies have been gained great attention in recent years. The nonthermal technologies such as high pressure processing (HPP), pulsed electric fields (PEF), ultrasound (US), UV-C light have emerged as alternatives to conventional thermal processing. The inactivation mechanisms of these treatments are not related to the heat effect. In order to consider a new technology as an equivalent of thermal pasteurization, the most resistant pathogenic microorganism for a certain food, the efficacy of the novel technology, the characteristics of the food product, the conditions needed for food distribution and storage, and intended use of food should be taken into account. Since some of these alternative technologies have been accomplished a decrease in the microbial population at a certain level and some of them have the potential application, the definition of pasteurization should be addressed in a more comprehensive way to meet with the standards for pasteurization (Barbosa-Cánovas & Bermúdez-Aguirre, 2011).

In this respect, the pasteurization term has been re-defined by USDA National Advisory Committee on Microbiological Criteria for Foods as: “Pasteurization is 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, 2004).

The thermal and some of the nonthermal processing technologies used for pasteurization of fruit juice are briefly summarized in next subsections. Fundamentals of selected nonthermal technologies are further discussed in Chapter 3.

2.4.2.1. Thermal Processing

Thermal pasteurization is the most widely applied preservation method to ensure food safety and extend the shelf life of food products. It is the traditional method which allows heating food products at temperatures below 100 °C. It can be defined as a way of destroying microorganisms of spoilage and public health concern through the application of a high-temperature for a sufficiently long time ensuring at least 5 log reductions of the most resistant microorganisms (FDA, 2001). This method is based on the heat which is generated from outside of a food and then transferred into the food through conduction and convection mechanisms (Pereira & Vicente, 2010).

Traditional thermal processing (pasteurization) can be performed either low temperature/long time (LTLT) or high temperature/short time (HTST). LTLT involves heating of food at about 63°C for no less than 30 min, while for fruit juices HTST pasteurization is conducted at temperatures of 72°C with holding times of 15 s and above (FDA, 2001).

Thermal processing emphasize on assurance of microbial safety. However, it can cause reactions that can affect taste, color, flavor and nutritional quality of foods (Awuah, Ramaswamy, & Economides, 2007). Degradation of heat sensitive vitamins such as Vitamins A (in the presence of oxygen), D, E, water-soluble Vitamin C (ascorbic acid), Vitamins B1 (thiamine), B2 (riboflavin) in acid environment (Ryley & Kajda, 1994); Maillard reaction which is a series of reactions between proteins and reducing sugars by means of Amadori rearrangements (Whistler & Daniel, 1985); protein denaturation covering alteration of quaternary, tertiary, secondary structures, and primary structures (Swaisgood, 1985); loss of color compounds including chlorophylls, anthocyanins, carotenoids, and betanins (Neilsen, Marcy, & Sadler, 1993) are the major changes developed in thermally treated food products.

Degradation of phenolics is one of the other adverse effects of thermal pasteurization. Thermal processing may cause complex physical and chemical reactions of phenolic composition, including the release of phenolic compounds from its bonded

forms, degradation of polyphenols, and the breakdown and transformation of phenolics (Nagy, Rouseff, & Lee, 1989). Due to such negative effects of thermal treatments on food constituents, innovative nonthermal technologies have been gained special attention.

2.4.2.2. Nonthermal Processing

Consumers have an increasing demand for fresh-like foods with high quality ensuring microbial safety, natural flavor, taste, nutrients, and bioactive compounds as well as less chemical preservatives (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007; Sanchez-Moreno et al., 2009). In addition to ensuring microbial safety, fresh-like characteristics and desirable sensorial attributes; nutritional and health related properties of juices are also required to be preserved in order to meet consumers' demands. Alternative methods have promising potential to replace the traditional processing methods by causing minor or no changes in the content of health-related phytochemicals (Sanchez-Moreno et al., 2009).

In this respect, nonthermal processing technologies such as HPP, US, PEF, and UV light have gained special attention as alternative methods to produce food with high nutritional and functional quality, and a longer shelf-life.

HPP has been investigated due to its ability to inactivate microorganisms and food enzymes while retaining or minimally modifying sensorial, nutritional, and health-promoting attributes of fruit and vegetable products (Cano & De Ancos, 2005; Hayashi, 1990). Application of multiple high pressure pulses and control of the end temperature around 105°C under pressure for a short time was proposed to achieve sterility with minimal effects on nutritional and health related constituents (Meyer, Cooper, Knorr, & Lelieveld, 2000). HPP is also referred to as high-hydrostatic pressure processing (HHP). The commercial use of HPP has been expanding. It provides food processors an opportunity to preserve foods with a "cleaner" ingredient label. It is the process of choice for applications where heat pasteurization can adversely affect product quality (Balasubramaniam, Farkas, & Turek, 2008)

US has been claimed as a promising technology in food processing due to its potential for allowing the improvement of existing processing as well as the development of new processing conditions (Knorr, Zenker, Heinz, & Lee, 2004).

Ultrasound refers to sound waves beyond the audible frequency range (in general, >20 kHz). When ultrasound passes through a liquid medium, the interaction between the ultrasonic waves, liquid and dissolved gas leads to an exciting phenomenon known as acoustic cavitation (AC) (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). AC has been addressed as the reason for microbial inactivation in foods. Since the power ultrasound has been identified as a potential technology that can meet the FDA requirement of a 5 log reduction of the microorganism of concern in fruit juices (Salleh-Mack & Roberts, 2007), it has gained attention to be an alternative promising technology for food processing applications. Even though no commercial food product processed by ultrasound technology is available yet, it has been in use with the combination of other technologies (Welti-Chanes, Barbosa-Canovas, & Aguilera, 2002). US provides benefits for the inactivation of microorganisms and enzymes due to its mechanical and biochemical effects generated by high intensity ultrasonic waves (Mason, Riera, Vercet, & Lopez-Buesa, 2005). Such benefits can be increased with the assistance of temperature and pressure.

PEF has a potential to replace the traditional method used for pasteurization of liquid food products such as milk and fruit juice and to increase the efficiency of extraction of components from plant foods (Deeth, Datta, Ross, & Dam, 2007). PEF processing is a treatment applying very short electric pulses (ms or μs) at high electric field strengths (kV/cm) and moderate temperatures (Martín-Belloso & Elez-Martínez, 2005a; Martín-Belloso & Elez-Martínez, 2005b) and results in destroying the functionality of cell membrane (Deeth et al., 2007). It can be applied to deliver safe and shelf-stable products such as fruit juice with “fresh-like” characteristics and high nutritional values. It has been demonstrated that PEF can achieve the equivalent microbial inactivation efficacy similar to thermal pasteurization and cause a much lower phenolic degradation compared to thermal pasteurization (Chen, Yu, & Rupasinghe, 2013).

UV-C irradiation being one of the alternative methods to traditional thermal treatment is based on inactivation of microorganisms at a wavelength between 200 and 280 nm (Koutchma, 2009a). Inactivation mechanism of UV-C processing is based on physical shifting of electrons and breaking of bonds in the deoxyribonucleic acid (DNA), thereby preventing the replication process, delaying of reproduction, and causing cell death. The high absorption of UV-C by DNA is associated with pyrimidine and purine bases’ ability to absorb light at 254 nm. Pyrimidine bases are particularly

sensitive to UV-C, inducing the formation of a covalent bond and resulting in a dimer formation. These thymine dimers inhibit correct replication of DNA during the cell's reproduction (López-Malo & Palou, 2005).

These technologies have been applied for many plant-based products including fruit juices (orange, pomegranate, apple, grape, berries), fruit juice-milk products and tomato juice (Rawson et al., 2011a; Sanchez-Moreno et al., 2009).

2.4.2.3. Hurdle Technology

Hurdle concept is a minimal processing approach for the gentle but effective preservation of foods. The hurdle effect is first highlighted in 1978 (Leistner, 1978). The microbial stability and safety of foods are achieved by a combination of several preservation factors (called hurdles) that the microorganisms present in food are unable to overcome. The preservation method based on more than one hurdle is designed to retain the natural and fresh-like properties of foods. In this context, hurdle technology accomplishes the interactions among preservation treatments. Combination of different technologies at their lower individual intensities have additive or even synergistic antimicrobial effects, while their impact on sensory and nutritive properties of the food is minimized (Ross, Griffiths, Mittal, & Deeth, 2003).

Although nonthermal technologies have been demonstrated to inactivate the spoilage or pathogen microorganism to varying degrees, individual applications of such technologies sometimes does not reach the desired inactivation levels, *i.e.* 5 log reduction in fruit juice, of target microorganism (Butz & Tauscher, 2002). Some nonthermal processing technologies has been claimed to be expensive and costly in terms of energy and practical use (Raso, Pagan, Condon, & Sala, 1998). Therefore, hurdle technology is a promising concept to overcome the drawbacks of individual technologies and create a synergistic or additive effect in a combined manner. This approach applies for two or more possible combinations of nonthermal technologies (*i.e.* HPP, PEF, ultrasound, ultraviolet light, pulsed light, ionizing radiation, oscillating magnetic fields) with each other as well as with lethal and sub-lethal heat treatments, lowered pH, and antimicrobial agents (Ross et al., 2003). Thus, an innovative strategy can be developed for the increase in antimicrobial effect of the preservation treatments by subjecting the microbial cell to a number of stresses (Leistner, 2000). Furthermore,

the hurdle concept leads to satisfy food preservation at lower individual treatment intensities (Barbosa-Canovas, Pothakamury, Palou, & Swanson, 1998).

The application of HPP at 827 MPa and 75 °C has resulted in an additional 2.5-log reduction of *B. subtilis* in the treatment buffer when the pH of the buffer was lowered pH from 7.0 to 3.0 (Balasubramaniam, Balasubramaniam, & Reddy, 2001). PEF processing at lowered pH values has also enhanced the microbial reduction. Liu et al., (1997) demonstrated that reduction of pH from 6.4 to 3.4 by using benzoic or sorbic acid were able to generate synergistic inactivation effect on *E. coli* O157:H7 in a 10 % glycerol solution treated with PEF (Liu, Yousef, & Chism, 1997). The synergistic effect of applying PEF at lowered pH values can be attributed to the enhancement of the entrance of undissociated acids into the bacterial cell which gained increased permeability due to PEF processing (Ross et al., 2003).

Gayan et al., (2012) claimed the difficulty of satisfying 5-log reduction standard for the inactivation of *E. coli* in UV-C treated turbid fruit juices with high absorption coefficients such as orange juice. Therefore, UV light was applied in combination with mild temperatures in order to overcome this problem. More than 5-log reductions of *E. coli* STCC 4201 suspended in orange juice was achieved when the juice was exposed to UV-C light at 55 °C, 57.5 °C and 60 °C (Gayan, Serrano, Monfort, Alvarez, & Condon, 2012). In another study, a blend of orange and carrot juice was subjected to PEF, UV light, or high intensity light pulses (HILP) in combination with manothermosonication (MTS) (400 kPa, 35 °C, 1000 W, 20 kHz) for the evaluation of several quality parameters. Sensorial analysis showed that color gained higher preference towards the juice treated by hurdle combinations rather than thermal pasteurization at 72 °C for 26 s. However, the flavor was adversely affected by the nonthermal technologies under the applied conditions (Caminiti, Noci, Morgan, Cronin, & Lyng, 2012a). Therefore, optimization of hurdle combinations should be carried out in order to avoid overprocessing. Accordingly, Caminiti et al. (2011) applied Ultraviolet light or HILP in combination with PEF or MTS to a blend of apple and cranberry juice. No significant change was found for the non-enzymatic browning, total phenolic content, and antioxidant activity of the blend. It was indicated that although UV+PEF and HILP+PEF applications did not affect the color, odor, and flavor of the product, HILP + PEF was able to better preserve the monomeric anthocyanins compared to other

combinations. The combined treatments including MTS negatively affected these properties (Caminiti et al., 2011).

Since an increase in temperature losses the membrane elasticity (Stanley, 1991) and reduces the thickness of lipid bilayer (Wu, Mittal, & Griffiths, 2005), the microbial cell can become more sensitive to PEF processing (Martin-Belloso & Sobrino-Lopez, 2011). Walking-Ribeiro et al. (2008) applied PEF (650 kJ/L, 34 kV/cm) at 55 °C which resulted in 7 log of *E. coli* in a tropical fruit smoothie (Walkling-Ribeiro et al., 2008). PEF processing of apple cider resulted in 5.9 log reduction of *E. coli* by applying EFI of 90 kV/cm and 10 pulses at 42 °C (Iu, Mittal, & Griffiths, 2001). In the same manner, another study on PEF processing (50 μs, 30 kV/cm) at 65-80 °C reduced the natural flora of orange juice-milk beverage by 5 log (Sampedro, Geveke, Fan, Rodrigo, & Zhang, 2009).

Thermal or nonthermal technologies can be also performed in combination with bacteriocin, enzymes, or essential oils. Espina et al. (2012) investigated the synergistic effect of citrus fruit essential oils combined with mild heat treatments and were able to achieve 5-log reduction of *E. coli* O157:H7 in sterile citrate-phosphate buffers (Espina et al., 2012). McNamee et al. (2010) achieved 5.6 log reduction of *Listeria innocua* in orange juice after processing by PEF (100 μs, 40 kV/cm) and addition of nisin (2.5 IU/mL) (McNamee et al., 2010). Hodgins et al. (2002) was able to lower the natural flora of orange juice by 6-log and 3.0-log when PEF treatment (20 pulses, 80 kV/cm) was applied in combination with nisin (100 IU/mL) and lysozyme (4,250 IU/mL), respectively (Hodgins, Mittal, & Griffiths, 2002). The natural flora of tomato juice was reduced by 4.4 log when the juice was subjected to PEF (20 pulses, 80 kV/cm) and nisin combination (Nguyen & Mittal, 2007).

Even though some innovative nonthermal processing technologies have found applications at industrial scale in food industry, many of them are still at their early stage of development. In order to guarantee the safety of final product, the mode of action, target elements within the microbial cell, as well as the treatment intensities required for cell inactivation should be taken into account (Ross et al., 2003).

CHAPTER 3

FUNDAMENTALS OF SELECTED NONTHERMAL PROCESSING TECHNOLOGIES AND FOOD APPLICATIONS

In recent years, food scientists and engineers have interested in investigating alternative food processing technologies to satisfy the consumer demands towards safe, nutritional, environment friendly and low cost products. Nonthermal processing technologies are gained attention due to the fact that their inactivation mechanism of microorganism is not related to the heat effect (Barbosa-Cánovas & Bermúdez-Aguirre, 2011). This chapter covers the fundamental concepts of some selected novel nonthermal food processing technologies. The mechanism of action, equipment and processing parameters, advantages and disadvantages as well as applications of each technology for fruit based products are assessed.

3.1. High Pressure Processing (HPP)

3.1.1. Basic Concept and Microbial Inactivation Mechanism of HPP

High pressure processing (HPP) is a “nonthermal” food preservation technology that uses high pressures to accomplish microbial inactivation or alteration of food attributes (Nguyen & Balasubramaniam, 2011). In order to obtain a microbiologically safe food, a typical range of 100–800 MPa, with or without the application of heat, has been used for inactivation of several pathogenic and spoilage vegetative bacteria, yeasts, molds, viruses, and spores (Balasubramaniam, Martiinez-Monteagudo, & Gupta, 2015). HPP has been also referred as high hydrostatic pressure (HHP), or ultra-high pressure (UHP) processing (Nguyen & Balasubramaniam, 2011). Main governing principles of HPP have been summarized accordingly.

Le Chatelier's principle

Le Chatelier's principle addresses that the pressure shifts the system equilibrium toward the state that occupies the smallest volume (Farkas & Hoover, 2000). Therefore, a system in equilibrium will shift to a new state of equilibrium due to a change in pressure. This principle has been used to explain the changes in the chemical, physical, and biological phenomena by the effect of pressure and temperature (Martínez-Monteagudo & Balasubramaniam, 2016). If any change in pressure (extensive variable) occurs, the equilibrium shifts in a direction that tends to reduce the change in the corresponding intensive variable (volume) (Balasubramaniam et al., 2015). In this respect, any phenomenon such as phase transition, change in molecular configuration, chemical reaction accompanied by a decrease in volume is enhanced by pressure (Hamann, 1957).

Isostatic principle

According to the isostatic principle, pressure applied to a fluid is equally transported through the contact surface. In HPP applications, a packed food material is surrounded by pressurizing fluid. The effect of pressure in the chamber is quasi-instantaneously and homogeneously distributed within the food regardless of food size and shape (Martínez-Monteagudo & Balasubramaniam, 2016). Since pressure is uniformly distributed throughout the entire sample, the processing time is independent of sample size and shape (Balasubramaniam, Farkas, & Turek, 2008).

Microscopic ordering

Principle of microscopic ordering indicates that, at constant temperature, an increase in pressure increases the degree of ordering of molecules of a given substance. Therefore, pressure and temperature exert antagonistic forces on molecular structure and chemical reactions (Balny & Masson, 1993).

Microbial Inactivation Mechanism

Currently, HPP is in use for processing of commercial solid or liquid food products. Development of novel HP treated products with or without heat is of great interest. Therefore, many researches have focused on the inactivation of microorganisms in order to obtain safe food with extended shelf life.

The microbial inactivation mechanism of HPP includes an alteration in the morphology of cell and disruption of cell membrane, ribosomal destruction, inactivation of enzymes, inactivation of membrane-bound transport systems, and damage to proton efflux system. HPP can cause homeostasis and denaturation of proteins including enzymes which have roles in the replication. These effects cause the impairment of cell functions, slowing the growth rate, and finally cell death occurs (Black, Stewart, & Hoover, 2011).

3.1.2. Equipment and Processing Parameters

During the last two decades, HPP has been used in food industry. Pre-packed products are treated in a chamber by using water or another pressure-transmitting fluid. Depending on the type of food product, the system can be either batch or semi-continuous (Balasubramaniam et al., 2008).

A typical HPP system consists of a pressure vessel; closure(s) for sealing the vessel; a device for holding the closure(s) in place while the vessel is under pressure; high-pressure intensifier pump(s); a system for controlling and monitoring the pressure and (optionally) temperature; and a product-handling system for transferring product to and from the pressure vessel (Balasubramaniam et al., 2008).

Figure 3.1. Hiperbaric 300 HPP System
(Source: http://www.hiperbaric.com/en/hiperbaric300)

Hiperbaric, Avure Technologies, Multivac are the major suppliers of HPP equipment. Figure 3.1 shows a hyperbaric HPP system with a vessel volume of 300 L and a diameter of 300 mm.

A high barrier and flexible pouch or a plastic container can be used as a packaging material. Once the packed food materials are loaded into HP chamber, the vessel is sealed and filled with pressurizing fluid. Pressure is increased by HP pump. The food packages surrounded by pressurizing fluid are then kept at the desired pressure for appropriate time. Then decompression by removing the pressurizing fluid follows (Farkas & Hoover, 2000) and finally the packed food products are unloaded. Figure 3.2 shows the processing line for HPP application.

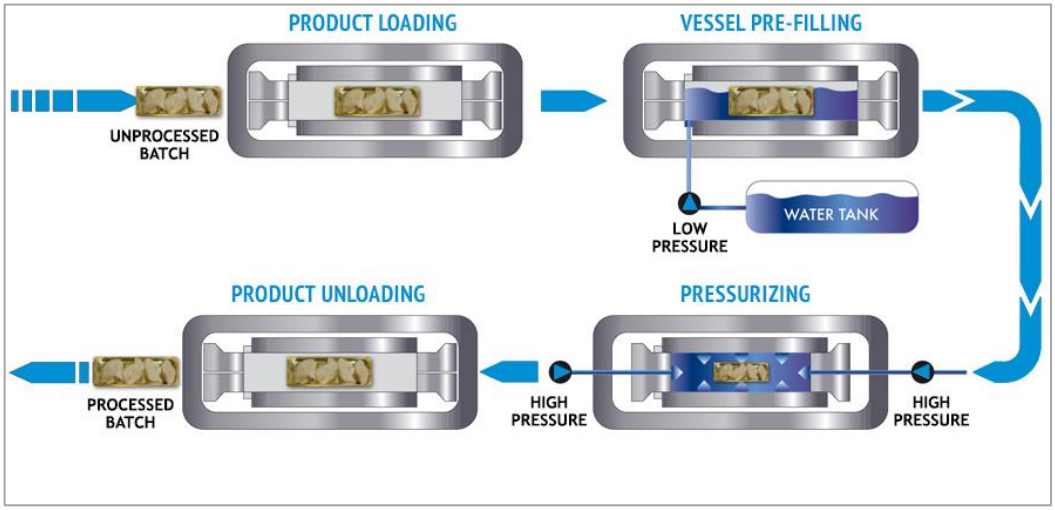


Figure 3.2. Processing line for HPP
(Source: http://www.hiperbaric.com/media/uploads/contenido/ficha/high-pressure.jpg)

Due to the thermodynamic effect of compression, the temperature of food product in HPP vessel increases during processing (Ting & Balasubramaniam, 2002). The work of compression during HPP treatment will increase the temperature of foods through adiabatic heating approximately 3 °C per 100 MPa, depending on the composition of the food. The temperature rise can be greater (8-9 °C/100 MPa) if the food contains a significant amount of fat (FDA, 2014). However, since rapid and uniform pressure transmittance is provided by high pressure throughout food, spatial variations are not appeared in high pressure applied products in comparison to heat, radiation, and microwave processes (Rasanayagam et al., 2003).

Figure 3.3 represents the change in pressure and temperature during high pressure processing. "t_2-t_1" is the time required to increase the pressure of the packed food product from the atmospheric pressure to the elevated target pressure. It is also called as "pressure come-up-time" (Farkas & Hoover, 2000). The come-up-time (CUT) depends on the target pressure, vessel volume, power of the pump-intensifier (Nguyen & Balasubramaniam, 2011).

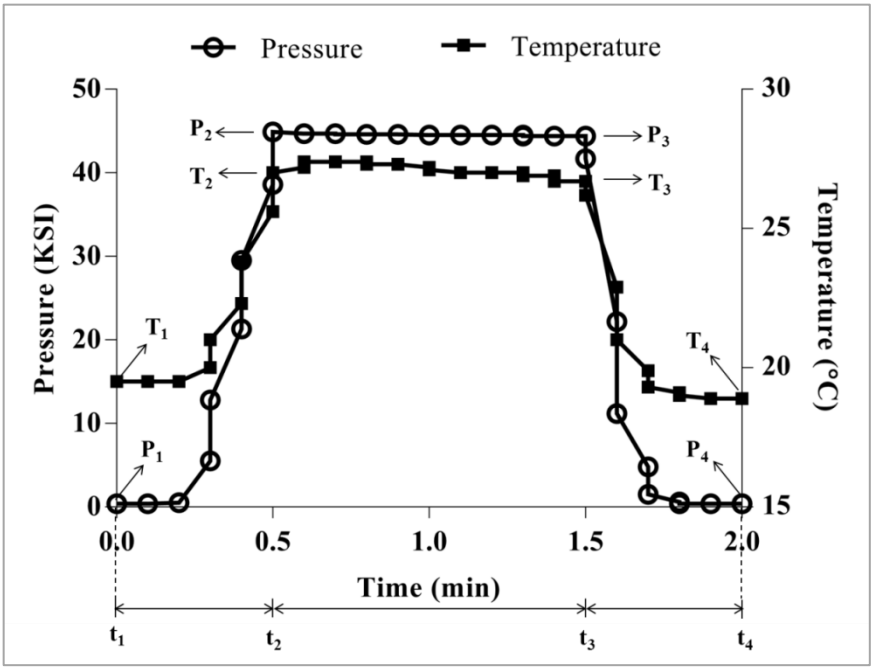


Figure 3.3. Pressure and temperature change during HPP

After reaching the target pressure, the system keeps the pressure at the desired level without addition of more energy. This period is called as the pressure holding time. Holding time can be defined as the time interval between the end of compression and beginning of decompression (t_3-t_2) (Figure 3.3). Depending on the insulation of the equipment, the temperature of product may decrease from T_2 to T_3 (Nguyen & Balasubramaniam, 2011). The holding pressure is also known as process pressure (Farkas & Hoover, 2000).

The time required to release the elevated pressure to atmospheric pressure is called as decompression time (t_4-t_3) (Figure 3.3) (Nguyen & Balasubramaniam, 2011). During decompression time, temperature of food product drops to a value lower than initial temperature. The difference between initial and final temperature is because of

the heat loss from the product to the surroundings during processing (Ting & Balasubramaniam, 2002).

The total time required for loading, closing the vessel, compression, holding, decompression and unloading is usually called as a cycle time (Nguyen & Balasubramaniam, 2011).

3.1.3. Advantages and Disadvantages

HPP offers several advantages in terms of product safety and quality, processing time, and novel product development. The advantages of HPP technology was summarized in Table 3.1. HPP allows food processing at ambient or even lower temperatures. Thus, any possible damage originating from the heating can be eliminated at the final product. Freshness, flavor, texture, and color of the product can be well retained by HPP compared to the traditional thermal processing. This technology can also eliminate the use of chemical preservatives or additives. With respect to the inactivation of spores, it can be possible to combine elevated pressures with heating in order to increase the lethal effect of processing. In this case, the processing is called as pressure assisted thermal sterilization (PATS). Since the pressure is transmitted throughout the vessel homogeneously and uniformly, the processing is independent from size and geometry of the product. This technology is applicable to both solid and pumpable foods. Due to the effect of pressure, some desirable changes can be achieved in the texture of food products such as shellfish. In-package processing avoids post-contamination after processing (Balasubramaniam et al., 2015; Fellows, 2009; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007).

Although HPP technology provides many advantageous, it also has some drawbacks (Table 3.2). The initial investment cost of HPP technology is a critical factor to be considered. However, since HPP has a positive consumer perception, the income can compensate for the capital cost. This technology is applicable as batch or semi-continuous form. It has less effect on reduction of enzyme activity. Another limitation is that the processed product should be kept at refrigerated conditions. Thermal non-uniformity can be seen when the pressure is combined with heating step (Balasubramaniam et al., 2015; Fellows, 2009; Rastogi et al., 2007).

Table 3.1. Advantages of HPP technology

Reference	Advantage of HPP
Rastogi et al. (2007)	Food processing at ambient temperature or even lower temperatures
	Instant transmittance of pressure throughout the system, regardless of size and geometry
	Microbial death by eliminating heat damage and the use of chemical preservatives/additives
Fellows, (2009)	Kills vegetative bacteria (and spores at higher temperature)
	No evidence of toxicity
	Color, flavor, and nutrients are preserved
	Reduced processing times
	Uniform treatment throughout food
	Desirable texture changes possible
	In-package processing possible
	Potential for reduction and or elimination of chemical preservatives
	Positive consumer appeal
Balasubramaniam et al. (2015)	Rapid and uniform distribution of pressure throughout the sample
	Minimal or reduced thermal exposure
	Instant temperature increase and subsequent cooling upon depressurization
	Suitable for both solid and pumpable foods
	Independent of product shape and size for processing time
	Opportunity for novel product formulation
	Within some pressure-thermal boundary conditions, pressure accelerates microbial inactivation
	Consumer acceptance as a physical process

Table 3.2. Limitations of HPP technology

Reference	Limitations
Fellows, (2009)	Less effect on enzyme activity Requires refrigeration of products. Expensive equipment
Balasubramaniam et al. (2015)	Batch or semicontinuous operation Preheating step for pressure-assisted thermal processing (PATP) Thermal nonuniformity during PATP Not suitable for products containing dissimilar compressibility materials such as marshmallows Variable efficacy in enzyme inactivation; pressure alone cannot inactivate bacterial spores Higher processing costs and batch operations are barriers for commodity product processing

3.1.4. Applications of HPP

Some recent studies relevant to the HPP treatment of fruit juices were summarized in this section. Bull et al. (2004) compared the microbial, physical and chemical properties of two types of orange juice subjected to HPP at 600 MPa and 20 °C for 60 s or thermal pasteurization carried out at different temperatures and processing times. Neither HPP nor thermal pasteurization (65 °C, 1 min) was able to achieve complete deactivation of pectin methyl esterase activity in Valencia orange juice (pH 4.3). However, PME was significantly reduced by HPP or thermal treatment (85 °C for 25 s) in Navel juice (pH 3.7) without significant alteration in quality parameters such as total soluble content, viscosity, titratable acidity, browning index, and color (Bull et al., 2004). On the other hand, ascorbic acid content and sensorial characteristics of orange juice were well retained by HPP (600 MPa, 40 °C, 4 min) compared to the thermal pasteurization conducted at 80 °C for 60 s (Polydera, Stoforos, & Taoukis, 2005). Hartyáni et al. (2011) also demonstrated the nonthermal preservation possibility of citrus juice products by HPP (Hartyani et al., 2011). Escobedo-Avellaneda et al. (2015) claimed that HPP resulted in an increase in ascorbic acid and some other compounds that can contribute to antioxidant activity of comminuted orange. This effect was suggested to be aroused from the cellular disruption mainly of flavedo by HPP and making them more accessible for quantification (Escobedo-Avellaneda, Gutierrez-Uribe, Valdez-Fragoso, Torres, & Welti-Chanes, 2015).

Queiroz et al. (2010) processed cashew apple juice by HPP at room temperature. Even though HPP at 250 or 400 MPa for 3, 5, and 7 min did not alter the physicochemical properties such as pH, acidity, total soluble solids, ascorbic acid or polyphenol content; 3 and 5 min treatment time of high pressure processing resulted in higher polyphenolic content (Queiroz et al., 2010). Chauhan et al. (2011) optimized HPP conditions for black grape juice by response surface methodology considering maximum retention of antioxidant activity, total phenolic and flavonoid content. The authors reported the optimum HPP conditions as 550 MPa, 44 °C, and 2 min (Chauhan, Raju, Ravi, Roopa, & Bawa, 2011). Torres et al. (2011) monitored the stability of anthocyanins and ascorbic acid in pressure treated blood juice samples during storage at 4 °C and 20 °C; and stated that the losses in anthocyanin and ascorbic acid content were significantly higher when the pressure treated juice kept at 20 °C rather than 4 °C. A

strong protective influence of ascorbic acid content on anthocyanin stability was claimed in the same study (Torres et al., 2011).

Chen et al. (2015) processed green asparagus juice by HPP at different pressures (200, 400, and 600 MPa) for 10 and 20 min as well as by thermal treatment at 121 °C for 3 min. The authors stated that HPP at 400 and 600 MPa and thermal treatment ensured microbial safety by achieving undetectable level of total mesophilic bacteria count. HPP was able to retain significantly more ascorbic acid, total phenolic content, and antioxidant activity than thermal processing (Chen et al., 2015). Gao et al. (2015) applied HPP (550 MPa for 10 min) or high temperature short time (HTST, 110 °C for 8.6 s) for the evaluation of microbial reduction and quality properties of red grapefruit juice. HPP was able to reduce total plate count and yeast-mold count by 4.83 and 4.15 log cycles, respectively. A longer shelf life and higher retention of total phenols, ascorbic acid, and antioxidant capacity were achieved for HPP-treated juice samples (Gao et al., 2015). Clear cucumber juice, clarified by ultra-filtration, was processed by HPP (500 MPa, 5 min) or HTST (110 °C, 8.6 s) and stored at 4 °C. Application of ultra-filtration was able to reduce total aerobic bacteria and yeast-mold count by 1.35 and 1.94 log cycles, respectively. Then, HPP or HTST reduced the total aerobic bacteria and yeast-mold count below 1 log cycle, and HPP and HTST treated samples did not show any further outgrow during 20 days of refrigerated storage (Liu, Zhang, Zhao, Wang, & Liao, 2016).

Huang et al. (2013) processed apricot nectar under high pressure (300-500 MPa for 5-20 min) or heat (HTST, 110 °C for 8.6 s). Even though HPP treatments increased the total and individual phenolic content in apricot nectars, the authors stated that the increase was significantly lower compared to HTST treated nectars. Moreover, HTST treatment was claimed to achieve complete inactivation of enzyme since polyphenol oxidase (PPO), peroxidase (POD), and pectin methylesterase (PME) showed resistance to high pressure inactivation. Thus, HPP was suggested to be accompanied by additional tools in order to increase enzyme inactivation (Huang et al., 2013). PME was claimed as the most resistant enzyme to HPP while PPO and POD showed similar sensitivity. Higher inactivation levels were achieved in mango pulp when HPP was applied at lower pH (Kaushik, Nadella, & Rao, 2015).

Patras et al. (2009) investigated phenolic compounds, antioxidant activity, and color of strawberry purée after processing by high pressure treatment (400, 500, 600

MPa/15 min/10-30°C) or thermal pasteurization (70 °C, 2 min). Pressure treated strawberry puree exhibited higher antioxidant activity and less color change compared to thermally treated samples. In the same study, HPP did not significantly change the anthocyanins; whereas a significant decrease was observed for thermally pasteurized puree (Patras, Brunton, Da Pieve, & Butler, 2009). In addition to individual phenolic compounds, enzyme (polyphenol oxidase, peroxidase, and β-glucosidase) inactivation was studied in strawberry pulps by Cao et al. (2011). The authors subjected the pulps to elevated pressures (400-600 MPa, 5-25 min) at room temperature. HPP at 500 and 600 MPa resulted in higher reduction of enzymes and better preservation of monomeric anthocyanins (Cao et al., 2011). In another study, strawberry puree samples from different cultivars such as Camarosa, Rubygem, and Festival was processed by HPP at 600 MPa and 20 °C for 5 min or thermal pasteurization (88 °C, 2 min). Although almost complete inactivation of polyphenol oxidase and peroxidase was achieved by thermal pasteurization, HPP resulted in 15–38 % and 20–33% inactivation, respectively. However, the authors claimed that a slightly higher loss was observed in total phenolic content and antioxidant capacity of pressure treated strawberry puree compared to heat treated samples after three months of refrigerated storage (Terefe et al., 2013). Cupped strawberry was subjected to HPP and thermal treatment following by storage at 4 °C and 25 °C. After evaluating microbial and nutritional quality, the authors concluded that HPP processing followed by storage at low temperature (4 °C) was useful for the preservation of cupped strawberry (Gao et al., 2016).

Wang et al. (2012) applied HPP for spinach puree considering pressure of 200, 400, and 600 MPa for 5, 15, and 25 min at room temperature. It was concluded that HPP treatment effectively prevented chlorophyll degradation and preserved a better visual green color (Wang et al., 2012). HPP was also applied to fruit smoothies. Keenan et al. (2012) compared thermal processing in retort and HPP in terms of nutritional quality of fruit smoothies. The authors claimed that thermal processing (P_{70} > 10 min) reduced the total anthocyanin and total phenolic content, ascorbic acid, lightness and redness color attributes of fruit smoothies compared to HPP at 450 MPa and 20 °C for 5 min. However, it is remarkable to indicate that there was a reduction in antioxidant properties as the pressure increased to 600 MPa (Keenan, Rossle, Gormley, Butler, & Brunton, 2012).

3.2. Ultrasonication (US)

3.2.1. Basic Concept and Microbial Inactivation Mechanism of US

Ultrasonication is one of the nonthermal processes alternative to existing conventional method. Ultrasound has been classified into two main categories: (1) power ultrasound, i.e., sound waves with lower frequencies and higher energies; (2) diagnostic ultrasound, i.e., sound waves with higher frequencies and lower energies (Weiss, Gulseren, & Kjartansson, 2011). Figure 3.4 shows the sound spectrum. Infrasound refers to the sound waves with a frequency of less than 20 Hz that is not detectable by human ear. The range of human hearing is between 20 Hz and around 20 kHz. Power ultrasound has a frequency range from 20 kHz to around 1 MHz. The diagnostic ultrasound has a frequency higher than 1 MHz which is mainly used for medical purposes (Kentish & Ashokkumar, 2011).

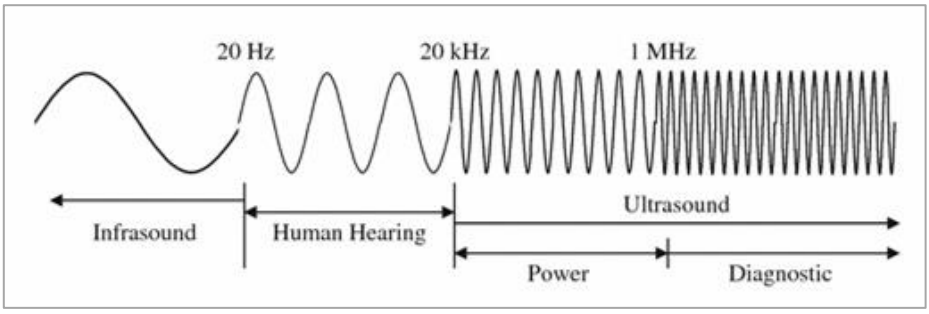


Figure 3.4. Representation of sound spectrum
(Source: Kentish & Ashokkumar, 2011)

Ultrasound travels through the medium; thereby creating compression and rarefaction of the medium particles due to a continuous wave-type motion (Knorr, Zenker, Heinz, & Lee, 2004; Rastogi, 2011). This phenomena is subsequently followed by formation of cavitation bubbles at sufficiently high power (Rastogi, 2011). The bubbles inside the liquid grow over the period of a few cycles. Once the bubbles reach to a critical size, they become unstable and then collapse violently (Mason, 1998). The implosion of cavitation bubbles results in energy accumulations in hot spot by generating extreme temperatures (5500 °C) and pressures (up to 50 MPa) (Leighton,

1998). This phenomena is called as cavitation (Leighton, 1995). Figure 3.5 shows the bubble formation and subsequent change during sonication.

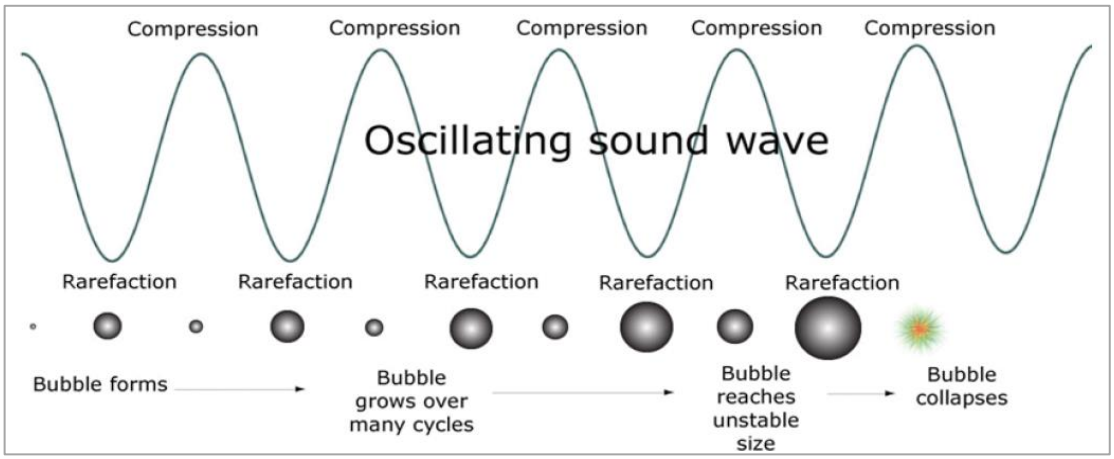


Figure 3.5. Bubble formation and subsequent changes over acoustic cycles (Source: Leong, Ashokkumar, & Kentish, 2011)

As a result of cavitation, extremely high shear energy waves and turbulence in the cavitation zone occurs. Thus, the characteristic of media can be altered by development of micro streaming currents and shear stresses resulted from bubble size variations and subsequent collapsing (Soria & Villamiel, 2010). The collapse of the bubbles generates the energy for chemical and mechanical effects (Mason, Riera, Vercet, & Lopez-Buesa, 2005). Depending on the applied sound power, different types of alteration can be seen in the media. Low or high energy can be used during sonication. Low energy ultrasound is frequently applied at frequencies higher than 100 kHz. It has applications in product assessment or control, ultrasonically assisted extractions, crystallization, emulsification, filtration, drying, and freezing, and tenderization of meat. High energy ultrasound, on the other hand, is usually applied at frequencies between 18 and 100 kHz for the purpose of degassing of liquids, extraction of proteins, inactivation of enzymes and microorganisms (Knorr et al., 2004).

Microbial inactivation is the major focus of this thesis. Ultrasonication provides a lethal effect on microorganisms by causing shear disruption, cavitation, thinning of cell membranes, and localized heating due to very rapid localized changes in pressure and temperature during processing (Leighton, 1998; Rastogi, 2011). The resistance of microorganism is dependent on shape and size of microorganism, type of cell, and physiological state. Bigger cells are more sensitive compared to smaller ones.

Regarding the shape of cells, coccal forms are more resistant than rod-shaped bacteria (Piyasena, Mohareb, & McKellar, 2003). The lethal effect on microbial inactivation can be enhanced by applying ultrasound under pressure (manosonication) or heat (thermosonication) or in combination with both pressure and heat (manothermosonication) (Rastogi, 2011).

3.2.2. Equipment and Processing Parameters

The first requirement for generation of ultrasound is a proper source of ultrasound which will be generated via an ultrasonic transducer. Transducer is a device which converts a type of energy into a different form. In the case of ultrasound, electrical energy can be converted to sound energy (Mason, 1998). The ultrasonic device is composed of a generator, ultrasonic converter, standard and booster horns, and probes as shown in Figure 3.6. The generator converts main electricity into the required high frequency alternating current to drive the transducer assembly (Mason, 1998). The ultrasonic converter, then, transforms electrical energy into mechanical vibrations of fixed frequency (Miguel Santos, Lodeiro, & Capelo-Martínez, 2009). In order to amplify the acoustic energy generated from a transducer, different types of horns are used (Mason, 1998). The standard and booster horns increase the sonication amplitude. The probes or detachable horns transmit ultrasonic energy into the sample (Miguel Santos et al., 2009).

The shape of the probes has an influence on the amplitude gain generated by the system (Mason, 1998). Figure 3.7 shows the ultrasonic probes in different shapes ((a) uniform cylinder; (b) exponential taper; (c) linear taper or cone; (d) stepped). D and d represent the diameter of driven face and the emitting face, respectively (Miguel Santos et al., 2009). In practical, the highest energy transferable from the source depends mainly on two factors: (i) the characteristic of materials for the construction of transducer assembly as well as the horn, (ii) the area of the emitting face. Regarding the material characteristics, highly resilient materials are required for the best transmission of the vibration. Small faced emitters can achieve the most efficient acoustic coupling. However, the transmission of the vibration can be limited at the surface when high amplitude cavitation bubbles forms (Mason, 1998).

It is important to have knowledge on the processing parameters in order to well design the sonication treatment. The parameters that have the influence on ultrasonication can be subdivided into three categories including acoustic factors (e.g. intensity of ultrasonic wave, frequency, pulse), medium characteristics (e.g. viscosity), and external factors (e.g. external temperature, external pressure) (Mason & Peters, 2002; Weiss et al., 2011).

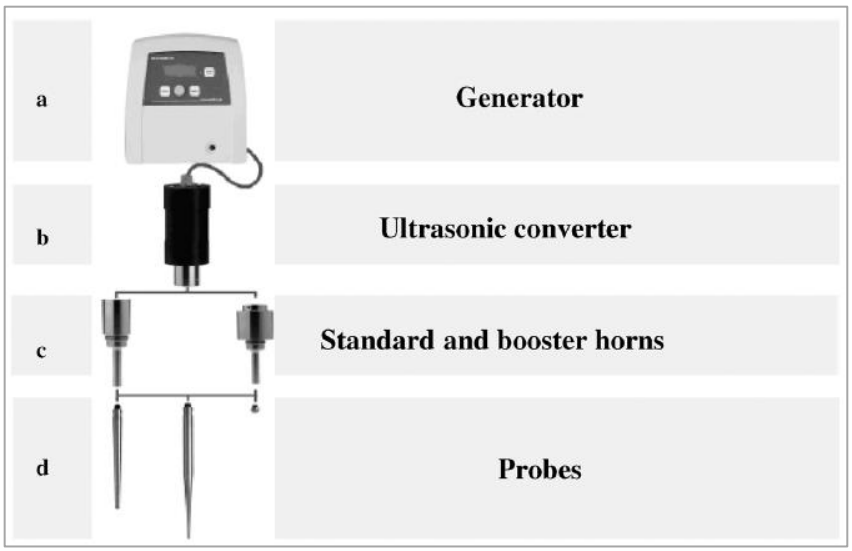


Figure 3.6. Parts of an ultrasonication device
(Source: Miguel Santos et al., 2009)

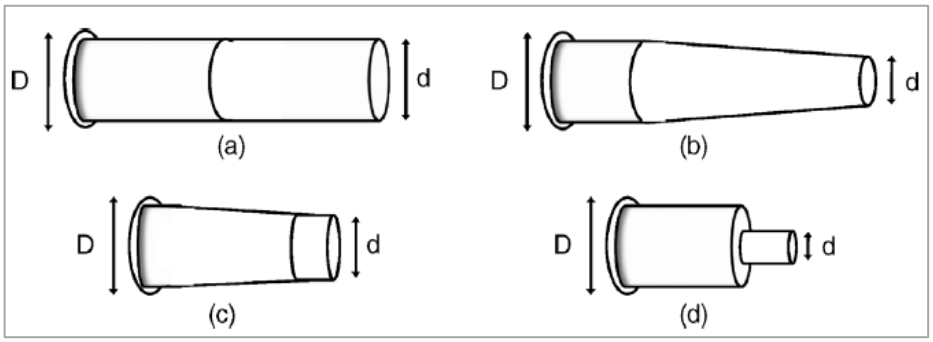


Figure 3.7. Ultrasonication probes in different shapes
(Source: Miguel Santos et al., 2009)

Acoustic factors

- **Intensity of ultrasonic wave**

The acoustic intensity must exceed a threshold value in order to produce cavitation. Higher intensity increases the sono-chemical effects; however, energy input

to the system cannot be increased indefinitely. Large amount of ultrasonic power generates a great number of bubbles which will coalesce further and avoid transfer of acoustic energy through the liquid by acting as a barrier (Mason & Peters, 2002).

- **Frequency**

Low frequency generates long acoustic cycle, thus large bubbles are created. On the other hand, acoustic cycle is short at high frequencies which results in smaller bubbles. As a consequence of smaller bubble formation, the cavitation collapse occurs less violently. Moreover, production of cavitation in liquids becomes difficult as the ultrasonic frequencies are increased (Mason & Peters, 2002).

- **Pulse**

The effect of cavitation may be altered considering pulse width, shape of wave form, and the interval between the pulses (Mason & Peters, 2002).

The influence of medium

- **Viscosity of the medium**

The generation of cavitation will be more difficult in viscous liquids where natural cohesive forces are large (Mason & Peters, 2002).

External factors

- **External temperature**

Use of external heating increases the temperature of the medium, thereby decreases viscosity and surface tension. Thus, a lower acoustic intensity is necessary for generation of cavitation since the cavitation threshold becomes lower. However, it should be taken into account that higher temperatures close to the boiling point of the liquid will generate a huge number of bubbles which can further reduce the transmission of ultrasonic energy from the source to the medium (Mason & Peters, 2002).

- **External pressure**

Applying an external pressure will require higher rarefaction pressure in order to initiate cavitation. Thus, a higher acoustic intensity will be necessary for the formation of cavitation bubbles compared to the applied intensity under atmospheric pressure (Mason & Peters, 2002).

3.2.3. Advantages and Disadvantages

Table 3.3 summarizes the advantages and disadvantages of US treatment. Ultrasound is mostly used in combination with other preservation technologies. The processing time can be reduced when ultrasonication is applied. It requires little adaptation to the existing processing plant due to operation either in batch or continuous systems. The heat transfer can be increased while running the sonicator. It can be effective for the inactivation of vegetative cells, spores, and enzymes (Fellows, 2009). Ultrasound and thermal processing result in synergistic effect with respect to microbial inactivation. Ultrasound assisted processing can provide quality improvements of liquid products due to degassing of the products (Knorr et al., 2004).

Table 3.3. Advantages and limitations of ultrasonication
(Source: Fellows, 2009)

Advantages
Effective against vegetative cells, spores, and enzymes
Reduction of processing times
Little adaptation is required for existing processing plant
Heat transfer increased
Batch or continious operation
Limitations
Complex mode of action
Depth of penetration is affected by solids and air in the product
Possible damage by free radicals
Undesired modification in food structure and texture
Needs to be used in combination with another process
Potential problems with scaling up

US has a complex mode of action. The local temperature differences can make it difficult to have a uniform heat distribution. In the same manner, cooling of the treatment unit may be also difficult to handle as the volume of sample increases. The depth of penetration of ultrasound inside the sample depends on the solid and air particles inside the product. Undesired modification in food structure can be observed.

However, this can be also an advantage depending on the type of product. For instance, the size of fat globules can be reduced by processing the milk with ultrasonication. Usually, ultrasonication needs to be used in combination with another technology in a hurdle approach. Scaling up for a larger volume production might have some potential difficulty and problems (Fellows, 2009)**.** Another challenge of ultrasound processing is the non-standardized reporting of methodology and control parameters. Due to the energy losses with increasing distance from the sound emitting surface, equipment improvement and changes in design should be carried out. Moreover, it is required to carry out a comprehensive development activities in order to understand, optimize, rigorously test and prove to be safe before being commercially available (Knorr et al., 2004).

3.2.4. Applications of US

Ultrasonication has been used in food processing for the purpose of crystallization of fats, sugars etc., degassing, destruction of foams, extraction of flavorings, filtration and drying, mixing and homogenization. In addition to those applications, its action on microorganisms and enzymes has been gained attention (Mason et al., 2005). Some other applications of ultrasonication in food engineering are reviewed by Chandrapala et al. (2012) considering emulsification, filtration, viscosity modification, dairy and tenderization (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). Ultrasound in combination with conventional or membrane filtration leads to efficient and longer operation without maintenance. Likewise, extraction of specific compounds can be enhanced by applying ultrasound assisted solvent extraction (Rastogi, 2011).

This section covers a review of current researches related to the microbial inactivation and preservation of quality parameters of fruits and fruit juices by ultrasound. Aadil et al. (2013) evaluated quality parameters of grapefruit juice subjected to US at 28 kHz and 20 °C. It was concluded that US processing improved total antioxidant capacity, ascorbic acid, total phenolic and flavonoid contents in the juice sonicated for 30, 60, and 90 min (Aadil, Zeng, Han, & Sun, 2013). Even though the authors suggested the use of US for grape fruit juice processing at industrial scale, long processing times may not be preferred for industrial applications. Sonication of apple

juice for 0, 30, and 60 min at 20 °C considering 25 kHz of frequency and 70 % of amplitude was evaluated by Abid et al. (2014). Results indicated that sonication for 30 min resulted in a significant increase in the content of polyphenolic compounds and sugar; whereas total carotenoids and some minerals such as Na, K, and Ca significantly increased after US processing for 60 min. The authors also reported losses in some mineral elements such as P, Mg, and Cu after sonication (Abid et al., 2014a).

Hurdle concept was developed for mango juice processing considering ultrasound (for 15 and 30 min at 25 °C, 40 kHz frequency) and UV-C treatment (for 15 and 30 min at 25 °C). The combined treatment led to a significant increase in extractability of carotenoids, polyphenols, and flavonoids along with the assurance of microbial safety (Santhirasegaram, Razali, & Somasundram, 2014). Martínez-Flores et al. (2015) applied ultrasound (24 kHz, 120 μm amplitude) in combination with thermal treatment (50, 54, 58 °C) for 10 min in order to evaluate microbial growth and quality parameters of carrot juice. Thermo-sonicated juice samples at 58 °C retained >98 % of carotenoids and 100 % of ascorbic acid while extending shelf life of carrot juice to 20 days at 4 °C. The authors, therefore, suggested thermosonication as a promising technology for preservation of carrot juice in terms of delaying microbial growth and retaining bioactive compounds (Martinez-Flores, Garnica-Romo, Bermudez-Aguirre, Pokhrel, & Barbosa-Canovas, 2015). Ultrasound (600 W, 20 kHz and 95.2 μm wave amplitude; 10 or 30 min at 20, 30 or 44 °C) was also used in combination with pulsed light for the inactivation of *Alicyclobacillus acidoterrestris* ATCC 49025 spores and *Saccharomyces cerevisiae* KE162 inoculated in commercial and natural squeezed apple juices. The spore reductions in commercial and natural apple juices were 3.0 and 2.0 log CFU/mL, respectively. Reduction of *S. cerevisiae*, on the other hand, was reported to be 6.4 and 5.8 log cycles in commercial and natural apple juices, respectively. US assisted with 60 s of pulsed light at the highest temperature build-up (56 °C) was claimed to be the most effective combination (Ferrario, Alzamora, & Guerrero, 2015). Different fruit and vegetable juices such as orange, sweet lime, carrot, and spinach juices were processed by ultrasound and ultraviolet irradiation. Results showed that the combination of ultrasound (frequency of 20 kHz, power of 100 W, 15 min) and UV-C light (2 UV-C lamps of 8 W) achieved an effective decontamination of juices as well as retained nutrient constituents to a higher level compared to thermal pasteurization (80 °C for 10 min) (Khandpur & Gogate, 2015).

Tiwari et al. (2008) subjected strawberry juice to ultrasonication considering ranging amplitude from 40 to 100 %, constant frequency of 20 kHz, treatment time of 2-10 min in pulse mode (5 s on and 5 s off). The authors were able to model the sonication effect on quality of strawberry juice by response surface methodology. It was concluded that the anthocyanin degradation was relatively low by ultrasound and compared favorably to thermal processing. Thus, it was suggested that ultrasound processing can be an alternative in juice preservation when the retention of nutritional quality is a priority (Tiwari, O'Donnell, Patras, & Cullen, 2008). In another study, Tiwari et al., (2009) observed that anthocyanins showed higher stability when the ultrasound treated strawberry juice was stored at refrigerated temperature 4 °C compared to 20 °C (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009a). Gani et al. (2016) applied US (33 kHz, 60 W) to freshly harvested strawberry fruits up to 60 min. The strawberries were then stored at 4 °C for 15 days after processing with US. Higher exposure time was reported to decrease the antioxidant potential and cause undesirable changes in the quality of fruit. 30 and 40 min of US exposure better preserved pH, vitamin C, and total soluble content. The authors stated that US processing for 40 min reduced the bacterial count from 5.91 log to 3.91 log CFU/mL (Gani et al., 2016). Bhat and Goh (2017) applied ultrasound for hand pressed strawberry juice. The physicochemical and phytochemical properties, as well as microbial load of juice were evaluated. Physicochemical properties such as pH, water activity, viscosity, titratable acidity, and turbidity were not significantly affected by sonication compared to untreated samples. Although sonication for 30 min enhanced bioactive compounds, it was not sufficiently effective on microbial inactivation under studied conditions (Bhat & Goh, 2017).

3.3. Pulsed Electric Fields (PEF)

3.3.1. Basic Concept and Microbial Inactivation Mechanism of PEF

Pulsed energy technologies utilize high energy levels in the form of short bursts or pulses. The energy is initially generated as electrical energy and accumulated in a storage capacitor following by discharging in the form of high intensity light pulses (pulsed light technology), high intensity magnetic field pulses (oscillating magnetic

field technology) or high energy electric field pulses (PEF technology) (Deeth, Datta, Ross, & Dam, 2007).

During the last decades, PEF has received remarkable attention due to its potential to enhance or develop quality retaining preservation processes as an alternative to conventional methods in food industry (Heinz, Alvarez, Angersbach, & Knorr, 2001; Toepfl, Heinz, & Knorr, 2005). PEF is a nonthermal processing method that applies direct current voltage pulses for very short periods of time, i.e. from microseconds to milliseconds, to a food material placed between two electrodes (Puertolas, Luengo, Alvarez, & Raso, 2012). Liquid food materials can be considered as electrical conductors due to the presence of ions which act as electrical charge carriers. The flow of electrical current in a treatment chamber generates a high voltage pulsed electric field within the food material (Zhang, Barbosa-Canovas, & Swanson, 1995). The electrical field causes permeabilization of cell membranes of microorganism present inside the treatment medium. The phenomenon related to the increment on the permeability of a cell membrane due to external electrical field pulses is called as electroporation or electropermeabilization (Puertolas et al., 2012; Tsong, 1991).

Exposure of a cell to an external field has the following effects (Saulo 2010):

- Increment in the transmembrane potential of the cytoplasmic membrane,
- Initiation of pore formation,
- Changes in the number of and/or size of formed pores during application of PEF,
- Acquisition of either reversible or irreversible electroporation.

Microbial inactivation mechanism is summarized in Figure 3.8. The cell membrane has been assumed as capacitor filled with a dielectric material of a very low dielectric constant in comparison with cell content and surrounding environment. Free charges accumulates at both membrane surfaces generating a transmembrane potential of about 10 mV (Zimmermann, Pilwat, & Riemann, 1974). When an external electrical field is applied, trans-membrane potential increases due to the additional free charges accumulating at both membrane surfaces. These charges are opposite and attract each other, resulting in membrane compression, and so the membrane thickness is reduced. When the transmembrane potential reaches approximately 1 V, the electro-compressive forces exceed the viscoelastic properties of the membrane and the membrane breakdown occurs (Pagán, Condón, & Raso, 2005; Zimmermann, 1986). Saulis (2010)

reported the critical value of external electrical field required for the induction of transmembrane potential as 0.2-1.0 V. Application of external electrical field close to the critical value forms reversible pores which can allow the cell membrane to recover its structure and functionality. PEF in the food industry requires irreversible electroporation in order to accomplish the microbial inactivation. Formation of irreversible pores results in disintegration of membrane and loss of cell viability when the applied electrical field is higher than the critical value (Saulis, 2010).

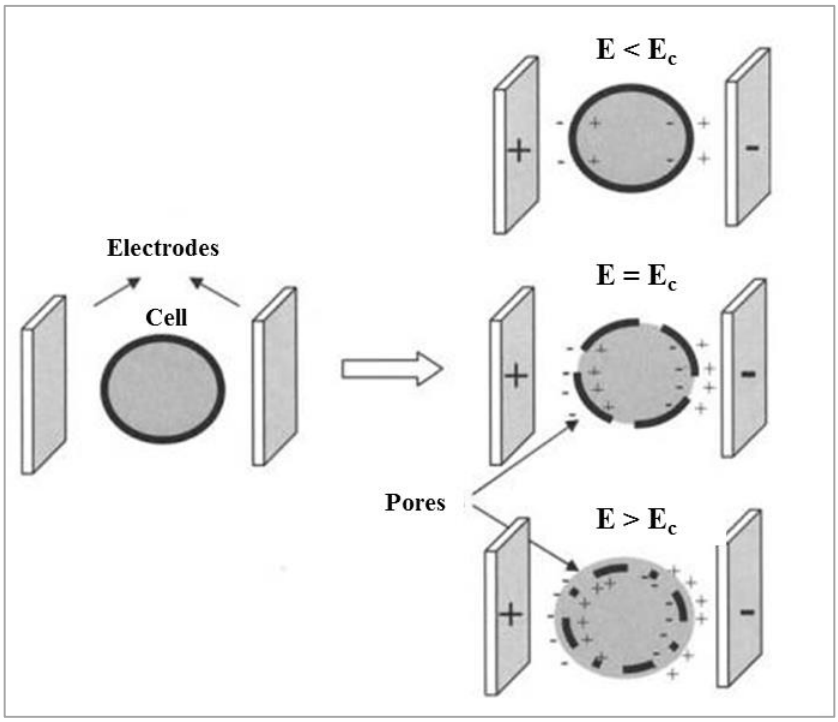


Figure 3.8. Mechanism of microbial inactivation by PEF treatment
(Source: Pagán & Mañas, 2006)

Heinz et al. (2001) reviewed the basic concepts of PEF. Regarding the microbial inactivation, the authors stated that the equilibrium of electrochemical and electrical potential differences of the cell plasma and extracellular medium is re-established due to the drastic increase in permeability. A simultaneous neutralization of transmembrane gradient across the membrane irreversibly impairs vital physiological control systems such as osmoregulation of the cell and finally cell death occurs. The authors also evaluated the relation between electrical field strength and cell geometry. The required electrical field increases as the characteristic dimension of the cell (*i.e.* the radius of spherical cells etc.) shifts to smaller values (Heinz et al., 2001).

3.3.2. Equipment and Processing Parameters

A pulsed electric field system composed of a high voltage pulse generator, pump, treatment chamber, and monitoring system. Figure 3.9 shows the flow chart of a PEF processing system. Continuous treatment chambers enable pumping the food through the system while the fluid is exposed to the electrical field at ambient or refrigerated as well as elevated temperatures. Heat exchangers can be used to heat the medium before or after the treatment. Besides, the dissipated electrical energy results in a temperature increase during PEF processing. The increased temperature has to be removed prior to packaging (Toepfl et al., 2005).

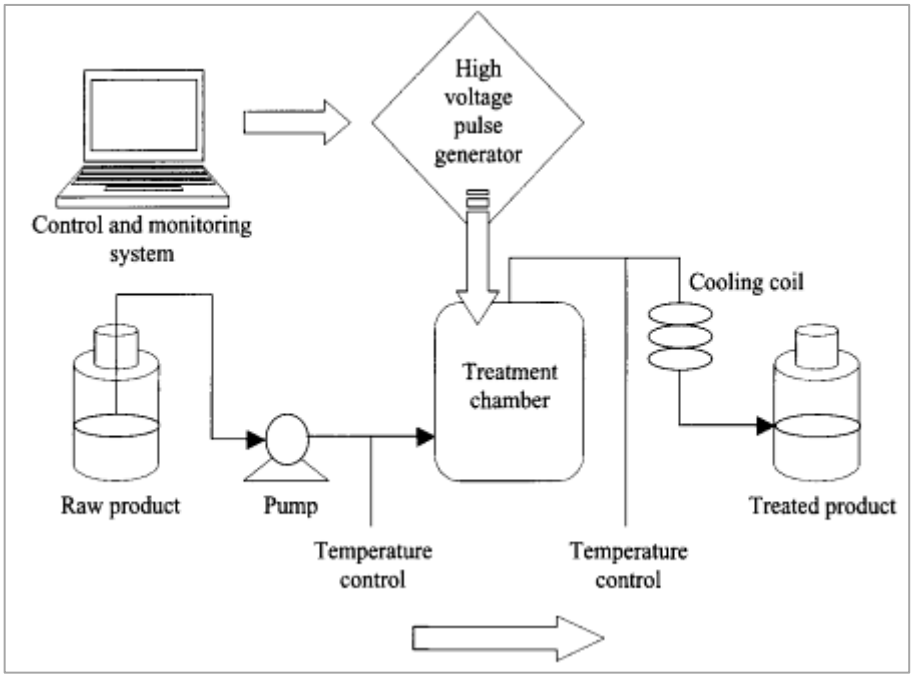


Figure 3.9. Flow chart of PEF processing
(Source: Barbosa Cánovas & Altunakar, 2006)

Several PEF parameters have been introduced in literature (Pagán et al., 2005):

Process Parameters:

- Electric field strength
- Treatment time
- Pulse characteristics
- Frequency
- Temperature

- Specific Energy

Microbial Characteristics:

- Intrinsic resistance of microorganisms
- Growth conditions
- Cell concentration

Product Parameters:

- Electrical conductivity
- pH
- Water activity
- Composition

The most commonly studied process parameters for the characterization of PEF technology are electrical field strength, pulse shape, pulse width, number of pulses, pulse specific energy, and frequency (Barsotti, Merle, & Cheftel, 1999). The strength of electrical field is defined by the distance between the treatment electrodes and delivered voltage as given the Equation 3.1:

$$E = \frac{V}{d} \tag{3.1}$$

where V is the voltage (kV) and d is the gap between electrodes (cm). Voltages used for the food treatment ranges between 10-60 kV (Toepfl et al., 2005). The treatment chamber consists of at least two electrodes in different geometric configurations, i.e. parallel plates, coaxial or co-linear cylinders, as shown in Figure 3.10. Although parallel electrode configuration provides a uniform electrical field in the chamber (Gerlach et al., 2008), the treatment intensity is reduced at the boundary conditions (Toepfl et al., 2005).

Three conditions for the accuracy of the electric field intensity have been defined (Bartels, 2001):

- The electrical field intensity must be homogeneous in the whole treatment chamber
- The residence time of the product must be uniform.

- The distribution of microorganisms throughout the product must also be uniform

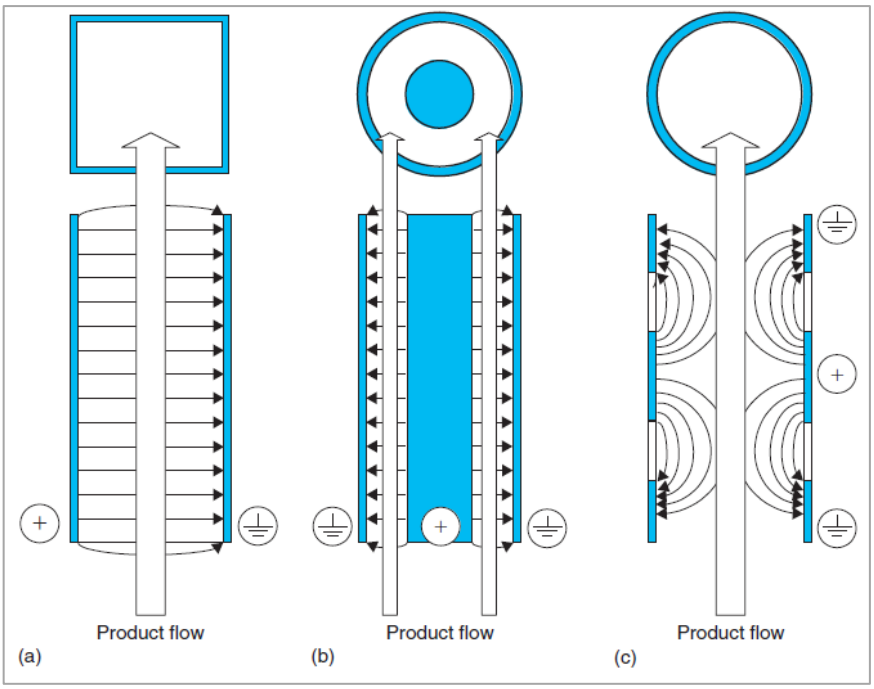


Figure 3.10. Configuration of PEF treatment chambers (a) parallel plate, (b) coaxial and (c) co-linear (Source: Toepfl et al., 2005).

Both the number and size of pores depend on the electric field strength and treatment time. During application, when the electric field reaches to values close to the critical electric field or when the treatment time is short, both the number and size of generated pores are low. Under these conditions, permeability of the membrane is reversible since the cell membrane restores its structure and functionality when the PEF treatment ceases. Application of an electrical field greater than the critical value results in an increase in the number and size of pores. Thus, irreversible permeabilization or mechanical disruption of the cell occurs (Pagán et al., 2005; Zimmermann, 1986). However, neither electrical field strength nor treatment time is enough to evaluate PEF processing. Other processing parameters such as wave form, pulse width, frequency, temperature, characteristics of media should also be taken into consideration.

PEF may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses and at ambient, sub-ambient, or slightly above ambient temperature for less than 1 s (Nip, 2007). Different types of electric field waveforms are shown in Figure 3.11. The most generally applied pulsed shapes used are exponential decay and square wave forms (Puertolas et al., 2012). The pulse width is defined as the

time where the peak field is maintained for square wave pulses or the time until decay to 37 percent for exponential decay pulses (Toepfl et al., 2005) as shown in Figure 3.12.

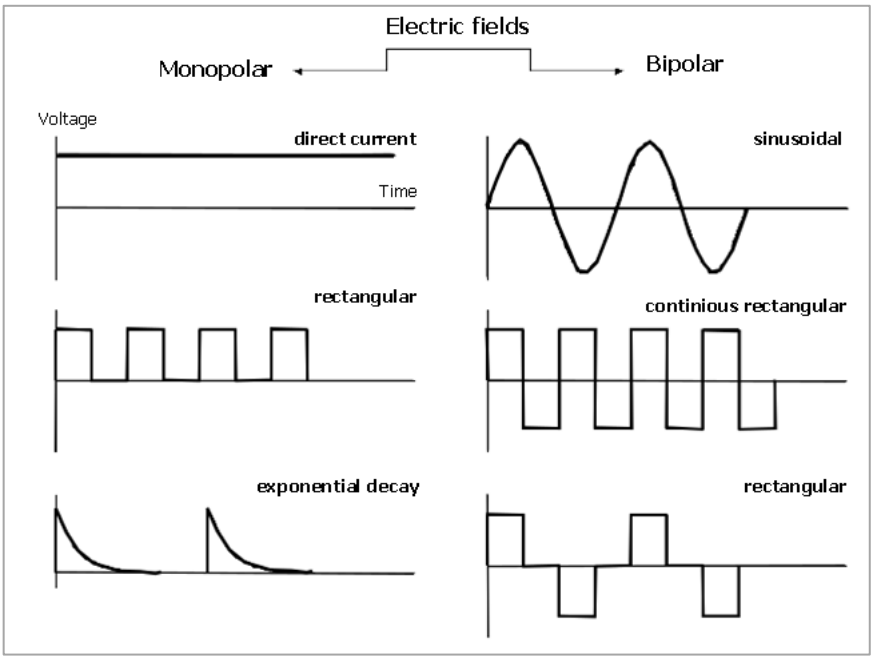


Figure 3.11. Different types of electric field waveforms
(Source: Ngadi, Bazhal, & Raghavan, 2003)

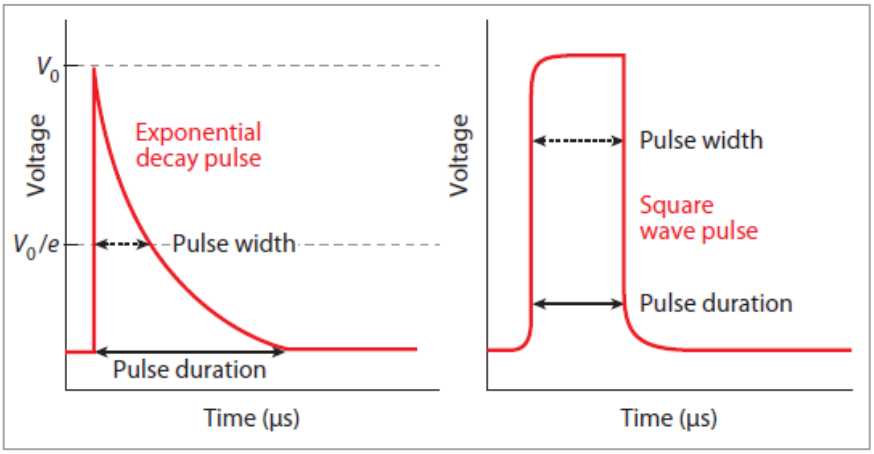


Figure 3.12. Pulse shape as a process parameter
(Source: Puertolas et al., 2012)

Since PEF treatment time is a function of duration of pulse width and the number of pulses applied (Puertolas et al., 2012), typically PEF treatment time increases with the increasing number of pulses (Toepfl et al., 2005). Thus, the treatment time is defined as:

$$t = n \times \tau \tag{3.2}$$

where n is the number of pulses, and τ is the pulse width (μs).

The specific energy of pulse is a function of applied voltage, treatment time, and the resistance of treatment chamber (Heinz et al., 2001; Puertolas et al., 2012; Töpfl, 2006) as given by Equation 3.3:

$$W_{specific} = f \cdot \frac{1}{m} \cdot \int_0^{\infty} \kappa(T).E(t)^2 dt \tag{3.3}$$

where *f* is the frequency (Hz), m is the mass (kg), κ is the electrical conductivity of media (mS/cm), E is the electrical field strength (kV/cm), and t is the treatment time. The frequency (or the repetition) can be defined as the number of pulses applied by unit of time. It can be expressed as Hz or pulses per second (Puertolas et al., 2012).

Treatment temperature has a synergistic effect on the efficacy of the processing. The temperature increase due to the dissipation of electrical energy can be utilized during processing (Töpfl, 2006).

Electrical conductivity (κ) is the inverse of the resistivity of material to electrical field and it can be expressed as Siemens per unit length (S/m). It is a function of temperature of media (Töpfl, 2006). Thus, the temperature increase will lead to changes in conductivity during treatment (Jayaram, Castle, & Margaritis, 1993). In addition to electrical conductivity, air bubbles and particles inside the media have a significant influence on the applicability of PEF. In the bubble regions, a significant drop can occur in the electrical field strength which can block the treatment and cause safety problems. Thus, the air bubbles have to be removed before processing (Töpfl, 2006).

3.3.3. Advantages and Disadvantages

PEF technology has gained interest in the field of food process engineering. Table 3.4 summarizes the advantages and disadvantages of PEF application. The continuous operability with very short processing times is one of the main advantages of PEF processing. Thus, a PEF system can be easily implemented into existing processing

lines (Toepfl et al., 2005). PEF processing can achieve the inactivation of vegetative cells by retaining color, flavor, and nutrients of the product in a relatively short treatment time (Fellows, 2009).

Table 3.4. Advantages and limitations of PEF
(Source: Fellows, 2009)

Advantages	**Limitations**
Kills vegetative cells	Less effective against enzymes and spores
Retains color, flavor, and nutrients	Only suitable for liquids
No evidence of toxicity	Safety concern in local processing
Relatively short treatment time	Energy efficiency is not certain yet

However, it is less effective on spores and enzymes. The continues systems are only suitable for pumpable fluids (Fellows, 2009). Before an industrial exploitation, it has to be demonstrated that the process is reliable enough to accomplish safe product, alternative to existing pasteurization methods in terms of cost of operation and investment. Moreover, product quality and consumer acceptance should be taken into account (Toepfl et al., 2005).

3.3.4. Applications of PEF

PEF assisted applications have been gained a strong interest in the field of food engineering since it offers several benefits over conventional ones. When a treatment such as extraction, drying or freezing is assisted with PEF technology, the mass transfer and extraction yield can be improved, as well as processing time and degradation of heat sensitive compounds can be reduced. PEF technology can assist extraction by diffusion and pressing, osmotic dehydration, drying, freezing, cold pasteurization (Barba et al., 2015).

Regarding the preservation of fruit juice products by PEF technology, the studies conducted to facilitate extension of shelf life and retention of quality parameters are summarized in this section.

PEF treatment was applied for the inactivation of different types of microorganisms in orange juice by McDonald et al. (2000). Electrical field strength of

30 kV/cm inactivated *Leuconostoc mesenteroides, E.coli, and Listeria innocua* in orange juice by as much as 5 log cycles at 50 °C (McDonald, Lloyd, Vitale, Petersson, & Innings, 2000). Sampedro et al. (2013) demonstrated that PEF processing of orange juice at 30 kV/cm and 60 °C reduced the population of *E. coli, Salmonella* Typhimurium and *Lactobacillus* spp. by more than 5 log, and was able to obtain extented shelf life up to 2 months at refrigerated condtions (4 °C) (Sampedro et al., 2013).

Agcam et al. (2016) subjected the orange juice to PEF or heat treatments. PEF processing resulted in better preservation of the juice quality compared to the heat treatment applied at 90 °C for 10 and 20 s (Agcam, Akyildiz, & Evrendilek, 2016). Elez-Martínez et al. (2006) compared high intensity pulsed electric field (35 kV/cm for 1,000 μs) and thermal pasteurization (90 °C for 1 min) for the shelf life extension of orange juice. PEF-treated orange juice samples showed microbiological stability up to 56 days at refrigerated conditions. However, the authors reported naturally occurring microorganisms within 30 days of storage at 22 °C. PEF treatment resulted in better retention of color compared to thermally pasteurized orange juice (Elez-Martinez, Soliva-Fortuny, & Martin-Belloso, 2006).

Aadil et al. (2015) processed grapefruit juice considering varying pulse electric field strengths up to 25 kV/cm at 40 °C for 600 μs. Phytochemical properties such as total phenolic and total anthocyanin content, total antioxidant capacity, total carotenoids as well as the physicochemical properties such as pH, total soluble content, titratable acidity, and color of the juice samples were evaluated. The authors suggested PEF processing of grapefruit juice at 25 kV/cm in order to improve the quality of the juice in terms of phytochemical and physicochemical properties (Aadil et al., 2015). Odriozola-Serrano et al. (2008) applied high intensity pulse electric fields (HIPEF, 35 kV/cm for 1700 μs, in bipolar mode at 100 Hz) and thermal pasteurization (90 °C for 60 or 30 s) to strawberry juice in order to evaluate bioactive compounds before and after processing. HIPEF-treated strawberry juice samples were found to be richer in the content of total anthocyanins as well as phenolic acids such as ellagic and *p*-coumaric acid compared to thermal treatment. Thus, PEF technology has been reported to be an effective technology to produce fruit juice with high antioxidant content (Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2008). According to the current perspectives, PEF

technology can be used for the development of environmentally friendly processes and new potential applications for food industry (Barba et al., 2015).

3.4. UV-C Irradiation

3.4.1. Basic Concept and Microbial Inactivation Mechanism of UV-C

Ultraviolet (UV) light occupies a range of wavelengths in the non-ionizing region of the electromagnetic spectrum. It is located between X-rays and visible light. Basically, it is subdivided into three regions as short-wave UV (UV-C), medium-wave UV (UV-B), and long wave UV (UV-A) (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). Figure 3.13 shows the ultraviolet components in the electromagnetic spectrum. Long-wave UV-A ranges from 315 to 400 nm, and it is normally responsible for tanning in human skin. UV-B varies from 280 to 315 nm that causes skin burning and can lead to skin cancer. Short-wave UV-C is between 200 and 280 nm that is called germicidal UV due to its inactivation power at a wavelength of 254 nm which is absorbed by most microorganisms' DNA (Koutchma, 2009a; López-Malo & Palou, 2005). A fourth region has been defined in the range of 100 and 200 nm. This region is called as vacuum UV range which can be absorbed by almost all substances and transmitted only under vacuum (Koutchma, 2009a).

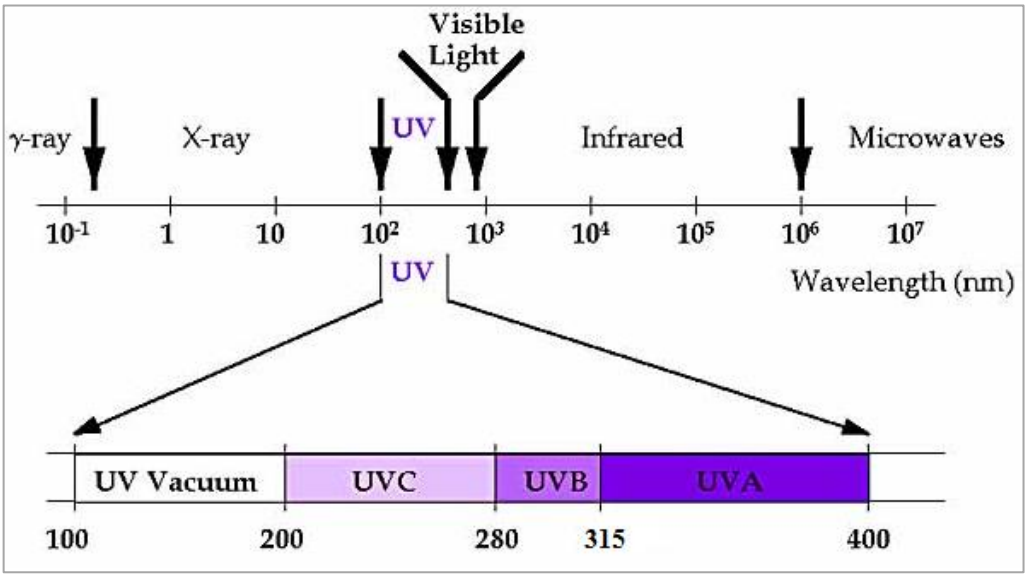


Figure 3.13. Ultraviolet component of the electromagnetic spectrum
(Source: Soehnge, Ouhtit, & Ananthaswamy, 1997)

The sun is the major source of a wide range of wavelengths. The UV radiation reaching to the earth's surface depends on the attenuation by the atmosphere via absorption and scattering. UV-A is barely affected from the atmosphere and the environment is exposed mainly to UV-A. UV-B is attenuated, however some UV-B can reach to the surface. On the other hand, UV-C is completely absorbed in the upper and middle atmospheres (Bintsis et al., 2000).

UV irradiation has found application for the disinfection of food products due to the germicidal effect of UV-C light. The absorption of UV-C light by DNA may stop cell growth and cause the cell death (Guerrero-Beltran & Barbosa-Canovas, 2004). Inactivation mechanism of UV-C processing is based on physical shifting of electrons and breaking of bonds in the deoxyribonucleic acid (DNA), thereby preventing life and reproduction. The high absorption of UV-C by DNA is associated with pyrimidine and purine bases' ability to absorb light at this wavelength. Pyrimidine bases are particularly sensitive to UV-C, inducing the formation of covalently bond resulting in dimer. These thymine dimers inhibit correct replication of DNA during the reproduction of cell (López-Malo & Palou, 2005). Figure 3.14 represents effect of UV light on DNA of a microorganism.

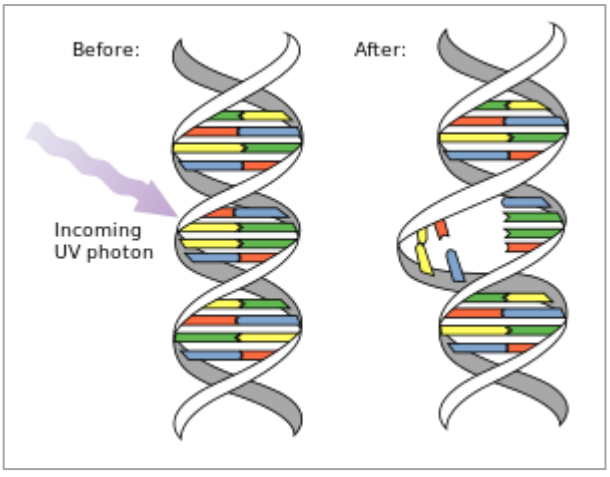


Figure 3.14. Mechanism of microbial inactivation by UV treatment
(Source: Flynn, 2015)

Among constituent bases of DNA, thymine undergoes a unique photochemical reaction. UV light damages the DNA of exposed cells by causing bonds to form between adjacent thymines in DNA chains. If two thymines are located adjacent to each other, the absorption of a UV photon by one of the bases forms a chemical bond causing

formation of a dimer (López-Malo & Palou, 2005). The resulting effect of the absorbed radiation by DNA is that the transcription and replication of DNA are blocked, cellular functions are obstructed, and eventually cell death occurs (Guerrero-Beltran & Barbosa-Canovas, 2004).

The influence of UV-C irradiation on the inactivation of microorganisms depends on the species, strain, growth media, and state of culture, load of microorganisms, food characteristics as well as processing parameters (Guerrero-Beltran & Barbosa-Canovas, 2004). The resistance of microorganisms to UV-C treatments is largely determined by their ability to repair DNA damage caused by UV. Gram negatives are the less resistant bacteria followed by Gram positives, yeasts, bacterial spores, molds, and viruses (Adams & Moss, 1995).

3.4.2. Equipment and Processing Parameters

UV-C processing can be applied either in continuous or discontinuous photoreactors. Discontinuous reactors are usually preferred when the medium has high viscosity or the need of high irradiation times. Continuous flow systems, on the other hand, achieve large quantum yields and small irradiation time (Falguera, Pagan, Garza, Garvin, & Ibarz, 2011). As shown in Figure 3.15, a typical photoreactor consists of a reactor, a reflactor, lamps, and a jacket (Falguera et al., 2011).

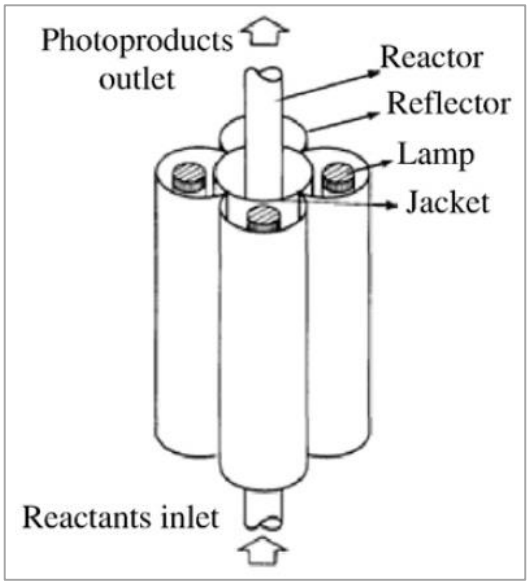


Figure 3.15. An example of UV-C processing reactor
(Source: Falguera et al., 2011)

UV-C processing has widely applied for liquid products. A typical UV-C system for processing liquids is composed of sample container, plastic tubing or sanitary pipes, pumps, reactor with UV lamps and jacket, refrigeration system (Guerrero-Beltran & Barbosa-Canovas, 2004) as shown in Figure 3.16. In order to achieve the required germicidal effect, the liquid can be passed through the system by re-circulating. A refrigeration system before or after UV-C light treatment can be used to cool down the liquid food (Guerrero-Beltran & Barbosa-Canovas, 2004). Mixing devices in the sample container lead to ensure the appropriate mixing of microorganism in the sample before treatment, and to obtain a representative sample for the evaluation of residual microorganisms after processing (Sastry, Datta, & Worobo, 2000).

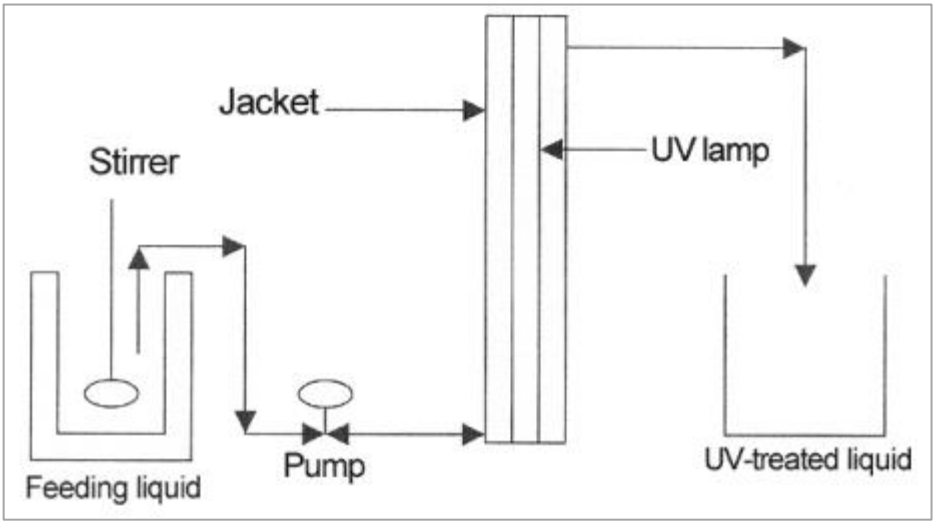


Figure 3.16. Diagram of UV-C processing system for liquids
(Source: Guerrero-Beltran & Barbosa-Canovas, 2004)

Factors affecting UV-C processing can be summarized as the type of UV irradiation source, ultraviolet dose, flow rate, lamp power, characteristics of liquid, type and number of microorganisms, growth stage of microorganisms, time, and particle content (Koutchma, 2009a).

As an artificial source of UV irradiation, different types of lamps can be used for UV processing of foods. Mercury vapor lamps, *i.e.* low pressure mercury lamps, low pressure high-output, and medium pressure mercury lamps, are used for UV applications (Koutchma, 2009a). The low and medium pressure mercury vapor UV lamps have been successfully used in water treatment for more than 50 years (Masschelein, 2002).

UV dosage deals with the irradiance, time and the volume of the reactor where the light penetrates into the liquid. The irradiance is generally expressed as watts per square centimeter (W/cm^2). For liquid products, UV dosage is usually given as J/L. The theoretical calculations related the intensity and dosage are given below (Keyser, Muller, Cilliers, Nel, & Gouws, 2008):

The average intensity (I) can be calculated as follows:

$$\text{Intensity } (I_{avg}) = \frac{\text{Total UV-C output per unit (W)}}{\text{Area } (cm^2)} \tag{3.4}$$

The retention time of the product inside the reactor can be calculated as below:

$$\text{Retention time (t)} = \frac{\text{Volume of the reactor (L)}}{\text{Flow rate (L/h)}} \tag{3.5}$$

UV Dosage for one reactor can be calculated as below:

$$\text{Dosage} = \text{Intensity (I)} \times \text{Time (t)} \tag{3.6}$$

UV dosage can be experimentally measured by biodosimetric and chemical actinometric methods (Rahn, 1997). The details of the measurement of actinometric method were given in Chapter 8.

It should be indicated that the required UV dose has a relationship with the characteristics of the treatment media. Juices which have high amounts of suspended particles need a higher UV dose compared to the clear juices. For instance, clear apple juice needs a lower UV dose to achieve effective reduction as expected, whereas orange juice and tropical juices with suspended matters require higher UV dosages in order to achieve the required reductions (Keyser et al., 2008).

3.4.3. Advantages and Disadvantages

Ultraviolet irradiation is a promising alternative to thermal processing in order to have a safe liquid with preserved quality (Falguera et al., 2011). So far, the technology has not been reported to leave any toxic or chemical by products (Keyser et al., 2008). It is effective against most type of microorganisms (Bintsis et al., 2000). In 2004, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of the USDA revised the definition of pasteurization and indicated that UV-C irradiation can be used as an alternative to heat treatments for pasteurization purposes (NACMCF, 2004). However, UV exposure has some limitations when treating juices. UV light only penetrates a very short depth in the juice when compared to clear water (Lu et al., 2010). Thus, the design of an UV-C equipment requires to take the radiation source, reactor geometry, and properties of medium into consideration (Falguera et al., 2011). Although it is safe to apply, some precautions are necessary to be taken in order to prevent the workers from exposure to UV-C light (Keyser et al., 2008).

3.4.4. Applications of UV-C

The practical applications of UV-C irradiation are divided into three categories: (i) inhibition of microorganisms on surfaces, (ii) destruction of microorganisms in air, (iii) sterilization of liquids (Bintsis et al., 2000). UV-C irradiation has been applied for many products including fruit juices, fruits and vegetables, fish, poultry, and meat (Guerrero-Beltran & Barbosa-Canovas, 2004). Regarding UV-C processing of fruit juices, several types of fruit juices such as orange juice, apple juice, guava-and-pineapple juice, mango nectar, strawberry nectar have been studied (Keyser et al., 2008; Tran & Farid, 2004). Recent studies in literature that has been attributed to microbial safety and product quality as affected by UV-C irradiation is given in this section.

Tran and Farid (2004) exposed freshly squeezed orange juice (FSOJ) to UV light as a thin film based on the idea of overcoming the difficulty of UV transmittance through the juice containing high suspended solids. It was reported that the shelf-life of FSOJ exposed to UV-C irradiation at 73.8 mJ/cm^2 was extended up to 5 days, whereas PME inactivation was not achieved by UV treatment. UV exposure of 100 mJ/cm^2 resulted in 17 % of vitamin C degradation which was similar to thermal treatment (Tran

& Farid, 2004). Gayan et al. (2012) combined ultraviolet irradiation with mild heat treatment for the inactivation of inoculated *E. coli* in orange juice. The authors used eight individual annular thin film reactors submerged in a water bath. Consequently, UV-C dose of 13.55 J/mL was able to achieve 5.41 log and more than 6 log cycles of inactivation of *E. coli* (STCC 4201) in orange juice at 57.5 and 60 °C, respectively (Gayan, Serrano, Monfort, Alvarez, & Condon, 2012). Pala and Toklucu, (2013a) stated that the log reductions for aerobic plate and yeast-mold count of natural flora, and inoculated *E. coli* (ATCC 25922) in orange juice were found to be 2.8 log, 0.34 log, and 5.72 log, respectively, after UV-C treatment (36.09 kJ/L). No significant differences was reported for untreated, UV-C and heat treated samples in terms of total phenolic content and antioxidant capacity (Pala & Toklucu, 2013a)

Apple juice is one of the extensively studied products subjected to UV-C processing. Caminiti et al. (2012) studied the effect of ultraviolet light on microbial inactivation and quality attributes of apple juice. They concluded that while UV energy levels were not affecting pH, °Brix, or total phenols content, it resulted in a decreased non-enzymatic browning ($p<0.01$) and antioxidant capacity ($p<0.05$) compared to unprocessed juice. All UV treatments applied at 2.66 J/cm^2 and above reduced the number of *E. coli* and *L. innocua* in apple juice below the detection level (<1 log CFU/mL) for both *E. coli* and *L. innocua* in apple juice (Caminiti et al., 2012b). Gayan et al. (2013) pasteurized apple juice inoculated with a cocktail of five strains of *E. coli* by applying UV-C in combination with mild temperature treatment. The optimum conditions that created synergistic effect and achieved more than 5 log reduction of the cocktail of five strains of *E. coli* were reported to be 27.10 J/mL of UV-C doses and treatment time of 3.58 min at 55 °C. Once the apple juice processed under these conditions, pH, °Brix, and acidity of the juice were not affected. The combination of UV-C and mild temperature almost doubled the inactivation of polyphenol oxidase (Gayan, Serrano, Monfort, Alvarez, & Condon, 2013). Tremarin et al. (2017) studied UV-C inactivation of *Alicyclobacillus acidoterrestris*, which is a thermo-acidophilic, non-pathogenic and spore-forming bacterium, in apple juice. The bacterial spores decreased linearly with the treatment time and reduced by approximately 5 log cycles when 13.44 W/m^2 of UV-C intensity was applied (Tremarin, Brandao, & Silva, 2017).

UV light was also applied on grape juice and wine by Fredericks et al (2011) resulting in an average log microbial reduction of 4.97 and 4.89 in Chardonnay and

Pinotage, respectively. In Chenin blanc and Shiraz juice, an average log10 reduction of 4.48 and 4.25 was obtained, respectively (Fredericks, du Toit, & Krugel, 2011). Pala and Toklucu (2013b) subjected white and red grape juices to UV-C light using nine lamps. UV-C dose of 12.6 J/mL in a single-pass was able to reduce the total aerobic plate count by 3.51 and 3.59 log and yeast-mold count by 2.71 and 2.89 log for white and red grape juice respectively. A complete elimination of microorganisms was achieved after circulation of juice products two times through the reactor (Pala & Toklucu, 2013b). Unluturk and Atılgan (2015) investigated the inactivation of *E.coli* K-12 (ATCC 25253) in freshly squeezed white grape juice by UV-C irradiation. The population of inoculated *E. coli* was reduced by 5.34 log cycles after circulating the juice 8 times through the system at a flow rate of 0.90 mL/s. Thus, UV-C exposure of 9.92 J/cm^2 led to extend the shelf life of freshly squeezed white grape juice up to 14 days at 4 °C without altering pH, total soluble content, and titratable acidity of the juice (Unluturk & Atilgan, 2015). Kaya and Unluturk (2016) processed clear and turbid grape juice in order to investigate the inactivation of inoculated *S. cerevisiae* and spoilage microorganisms (yeasts and lactic acid bacteria) by UV-C irradiation in a continuous flow system. While 65.50 mJ/cm2 of UV dose was able to reduce the population of *S. cerevisiae* by 3.39 log cycles, comparatively lower amount of reductions were obtained for turbid grape juice at higher UV doses. The reductions for yeasts and lactic acid bacteria in turbid juice were reported to be 1.54 and 1.64 log CFU/mL, respectively (Kaya & Unluturk, 2016).

Pala and Toklucu (2011) subjected pomegranate juice (PJ) to UV-C irradiation. It was declared that UV-C treatment preserved the major quality characteristics of pomegranate juice better than heating process by comparing UV-C treated juice with heat treated (at 90°C, 2 min) and control. After UV-C treatment, total monomeric anthocyanin content of pomegranate juice did not change significantly and decrease in individual anthocyanin pigments were between 8.1% and 16.3%. However, individual anthocyanin pigments of PJ were significantly affected by heat treatment ($p < 0.05$) and decreased in-between 15.4% and 28.3%. The effectiveness of the UV-C system on the aerobic plate count, yeast and mold count and *E. coli* ATCC 25922 as a surrogate microorganism of *E. coli* O157:H7 in PJ resulted in 1.8 log, 1.45 log and 6.15 log reductions, respectively (Pala & Toklucu, 2011). Santhirasegaram et al. (2015) processed mango juice by UV-C treatment (15, 30, and 60 min at 25 °C) and thermal

pasteurization (90 °C for 60 s). The authors observed an increase in content of carotenoids (6%), polyphenols (31 %) and flavonoids (3 %) after UV-C irradiation for 15 and 30 min treatment time compared to untreated samples. The shelf life of mango juice was extended 4 weeks more than the freshly squeezed juice. Although a complete inactivation of microbial growth was satisfied with the thermal pasteurization, the quality was adversely affected by heat (Santhirasegaram, Razali, George, & Somasundram, 2015a). Kaya et al. (2015) achieved more than 6 log CFU/mL reduction of inoculated *E. coli* in lemon-melon juice blend by UV-C irradiation (2.46 J/mL) and thermal pasteurization (72 °C, 71 s). The authors reported that UV-C irradiation resulted in better preservation of physicochemical properties of the blends compared to heat treatment (Kaya, Yildiz, & Unluturk, 2015).

CHAPTER 4

MATERIALS AND METHODS

This chapter covers the general methods used for the studies conducted both at Washington State University (Chapter 5-7) and Izmir Institute of Technology (Chapter 8 and 9). Other methodologies specific to each chapter are given in the "Material & Methods" section of the corresponding chapters.

4.1. Raw Material

4.1.1. Freshly Squeezed Strawberry Juice

The preparation of strawberry juice used for the studies given in Chapter 5, 6, and 7: Strawberries (Fragaria × ananassa) were purchased from a grocery store (Walmart Supercenter, Pullman, USA) at their commercial maturity. Fruits were stored at -30 °C prior to use. The fruits were defrosted by leaving them overnight at ambient temperature in dark. Then, the juice was extracted using an electric centrifugal juicer, i.e. a fruit juice extractor. Next, the juice was centrifuged (Beckman J2 HS centrifuge, GMI, MIC Group, Inc., Minnesota, US) at 6000 rpm and 4 °C for 5 min using a Fiberlite F14 6x250 rotor (Piramoon Technologies, Inc., US). The juice was subsequently filtered through a cheese cloth to remove the suspended particles.

Preparation of strawberry juice for the studies outlined in Chapter 8 and Chapter 9: Strawberries at commercial maturity were obtained from a local bazaar in İzmir. Fruits were stored at -20 °C until processing. Frozen fruits were then defrosted overnight at ambient temperature in dark. Strawberries were squeezed using a household table top juice extractor (Arçelik, İstanbul) and subsequently centrifuged (Allegra 25R, Beckman Coulter, Indianapolis, US) using a fixed angle rotor (TA-10-250, Beckman Coulter) at 10000 rpm for 5 minutes. Filtration through a sterile gauze strip was applied to remove any possible particles.

4.1.2. Pre-pasteurized Strawberry Juice

Freshly squeezed strawberry juice was pasteurized by thermal treatment in order to reduce the initial microbial load of juice. Pasteurization time and temperature parameters were chosen considering the study of Timmermans et al. (2011) and FDA's pasteurization criteria (FDA, 2004; Timmermans et al., 2011). The background microflora of SJ was eliminated by heating the juice at 71.7 °C for 15 s. For this purpose, SJ was put into a double walled sample container connected to a water bath (Viscotherm VT 10, Paar Physica, Germany) (Figure 4.1). The sample unit was placed on a magnetic stirrer. Hot water (74 °C) was circulated between the walls of sample container. A magnetic stirrer bar was used in order to increase the heat transfer in the medium. Temperature was controlled by a K-type of thermocouple. Once the temperature reached 71.7 °C, the juice was kept for 15 s and then transferred to a previously sterilized bottle. Finally, the treated juice was cooled down to room temperature by placing in ice. The juice free from the initial background microflora was re-named as **"pre-pasteurized strawberry juice"** and used for studies in which a target non-pathogenic microorganism was inoculated and inactivated by selected technologies described in Chapter 5-7.

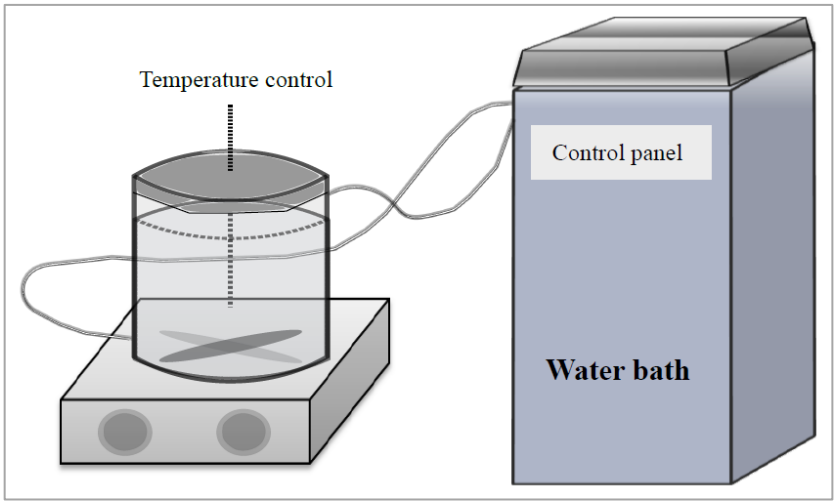


Figure 4.1. Schematic view of thermal pasteurization unit used at WSU

4.2. Measurement of Physicochemical Properties

4.2.1. pH

pH measurement was carried out by using a bench top pH meter (FE20 FiveEasy, Mettler Toledo™, USA and inoLab pH 7310, WTW GmbH, Weilheim, Germany) at 22°C. Ten mL of SJ was used for the measurement. The probe of pH meter was directly immersed into the sample and the reading was recorded when the pH value reached to a constant value Calibration of pH meter was carried out by using two buffer solutions at pH 7 and pH 4.

4.2.2. Titratable Acidity

The amount of titratable acidity of SJ was determined by titrimetric method (Cemeroglu, 2010). Ten mL of sample was poured into a flask and titrated against standardized 0.1 N NaOH up to pH 8.1 which is phenolphthalein end point. The amount of NaOH used for titration to reach pH 8.1 was recorded. Since the major organic acid in SJ was citric acid, the amount of titratable acidity was expressed as g citric acid per 100 mL strawberry juice using the following equation:

$$\text{TA (g/100 mL)} = \left(\frac{(V)(f)(E)(100)}{(v)} \right) \quad (4.1)$$

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

4.2.3. Total Soluble Solid (TSS)

A digital hand-held refractometer (PAL-α, Atago CO., LTD.) was used to determine the total soluble solids (°Brix) of the samples. The refractometer was firstly adjusted to zero by using distilled water. Then, 3-4 drops of strawberry juice were

poured into the measuring cell of refractometer. Before measurement, the refractometer was adjusted to zero by reading the TSS of distilled water. Measurements of TSS were conducted at ambient temperature in triplicate.

4.2.4. Turbidity

Turbidity of strawberry juice samples was measured by using a turbidimeter (HACH 2100AN IS, Loveland, CO, USA). The outer surface of sample cell was cleaned with silicone oil and oiling cloth to avoid the light scattering. The sample cell containing 30 mL of SJ was placed into the sample compartment. Turbidity was measured in Nephelometric Turbidity Units (NTU).

4.2.5. Absorption Coefficient

Absorption coefficients of strawberry juice samples were determined by using a UV-Visible spectrophotometer (Varian Cary 100, Agilent Tech., Santa Clara, CA, USA). Several dilutions of SJ samples were prepared with distilled water at varying ratio of 1:25, 1:50, 1:100, 1:200, and 1:500. Then, the absorbance of diluted samples was measured at 254 nm using quartz cuvettes with a path length of 1 cm. The absorption coefficient of SJ was estimated from the slope of the absorbance-juice concentration plot.

4.2.6. Color

CIE refers to the French title of International Commission on Illumination (Commission Internationale de l´Eclairage). The CIE system defines the color in three-dimensional color space, i.e. L* (brightness-darkness), a* (redness-greenness), b* (yellowness-blueness) (MacDougall, 2002). L* value shows the degree of lightness ranging from black to white based on a scale from 0 to 100. Increasing L* values indicate the increase in lightness. Larger values of a* refers to an increase in red; whereas larger –a values indicates an increase in green. On the other hand, greater +b and -b values shows the increase in yellow and blue, respectively (Lawless & Heymann,

2010). CIE L*, a*, b* values of the samples were measured by a Chroma meter (CR 400, Konica Minolta Inc., Japan). Thirty mL of treated and untreated SJ samples were placed into a quartz container. The measurement was conducted at three different points of the samples in duplicate. Calibration of the equipment was conducted by using a white reference plate by adjusting Y, x, and y values to 93.8, 0.3159, and 0.3322, respectively.

Total color difference (ΔE) (H. S. Lee & Coates, 1999) and browning index (Palou, Lopez-Malo, Barbosa-Canovas, Welti-Chanes, & Swanson, 1999) of the samples were calculated from Equations 4.2 and 4.3, respectively.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{4.2}$$

where the sign Δ shows the difference between untreated and treated juice samples regarding the corresponding color parameter.

$$BI = 100 * \frac{\frac{\left(a^* + 1.75L^*\right)}{\left(5.645L^* + a^* - 3.012b^*\right)} - 0.31}{0.172} \tag{4.3}$$

Hue (h*) and C* (Chroma) values of the samples, on the other hand, were calculated by using a* and b* values as shown in Equation 4.4 and Equation 4.5.

$$h^* = \arctan\left(\frac{b^*}{a^*}\right) \tag{4.4}$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \tag{4.5}$$

The color wheel in Figure 4.2a shows the continuous spectrum of colors, i.e. hue, which is perceived by human eyes as the object's color (e.g. red, orange, green, etc.) (X-rite, 2007). h* is the angle that is expressed in degrees. As shown in Figure 4.3, 0° and 360° refer to +a (red), 90° refers to +b (yellow), 180° is –a (green); while 270° is –b (blue) (Lawless & Heymann, 2010; X-rite, 2007). Chroma describes the vividness or dullness of a color (Figure 4.2b). As an indication of color saturation, C* is equal to

zero at the center of color space and increases as the distance changes towards 60. It can be also defined as the degree of visual difference from neutral grey (Lawless & Heymann, 2010; X-rite, 2007).

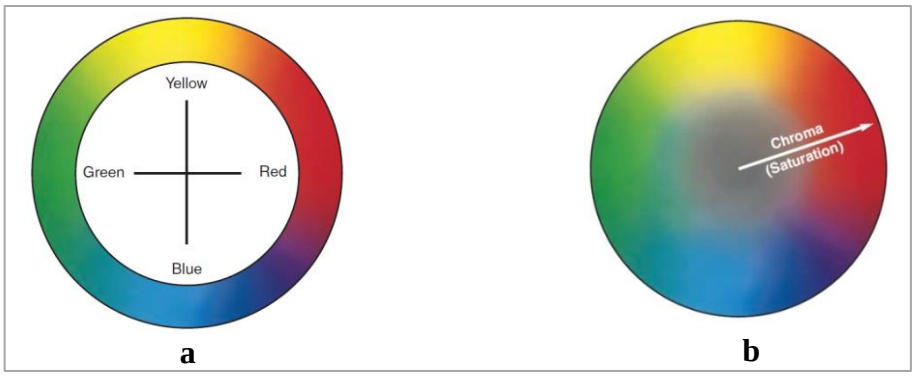


Figure 4.2. Color wheel (a) and Chromaticity (b)
(Source: X-rite, 2007)

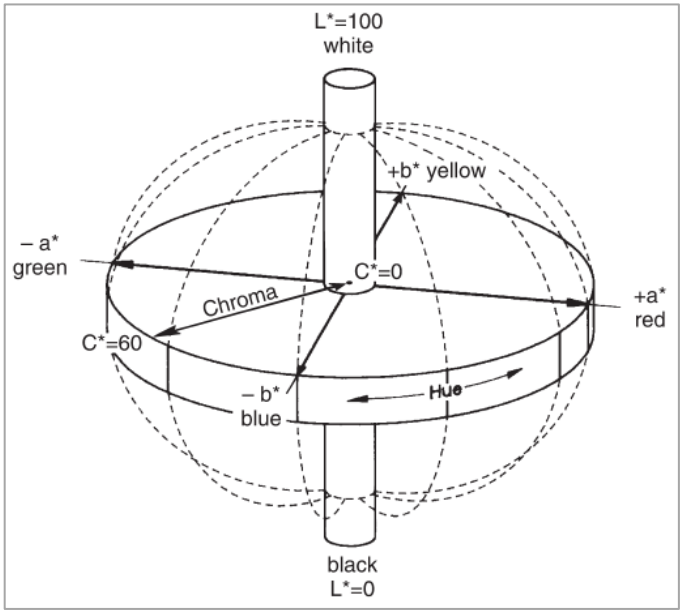


Figure 4.3. Three dimensional color system
(Source: X-rite, 2007)

4.3. Measurement of Phytochemical Properties

4.3.1. Total Phenolic Content (TPC)

Total phenolic contents of SJ samples were determined by using Folin-Ciocalteu method (Singleton & Rossi, 1965; Tezcan, Gultekin-Ozguven, Diken, Ozcelik, & Erim, 2009). SJ samples were firstly diluted in the ratio of 1:100 by using MeOH:H_2O (3:2). 300 μL of diluted SJ mixed with 1.5 mL of Folin–Ciocalteu reagent (diluted 10 fold with distilled water) in a glass tube followed by the addition of 1.2 mL of sodium carbonate (75 g/L) considering a time interval up to 5 min. The mixture was thoroughly mixed by using vortex mixer (ZX3, VELP Scientifica S.r.l., Usmate, Italy) and allowed to stand for a further 90 min in dark and the absorbance was measured at 760 nm using a HP 8452A diode array spectrophotometer (Agilent Technologies, Palo Alto, USA). The solvent MeOH:H_2O (3:2) was used to adjust the zero absorbance prior to measurements. A control sample tube (blank) was prepared by using 300 μL of MeOH:H_2O (3:2) with the absence of SJ. The absorbance of control sample was then subtracted from the absorbance of juice samples. Calibration was performed using gallic acid standard solutions prepared at different concentrations using a stock solution (1000 ppm). The same procedure was also applied to gallic acid standard solutions at varying concentrations up to 150 ppm. The concentrations of total phenolic compounds in SJ samples were expressed as mg gallic acid equivalents (GAE) per L.

4.3.2. Total Anthocyanin Content (TAC)

Anthocyanin pigments undergo a structural change and form different colors at different pH values leading to differences in absorbance readings. The change in absorbance directly correlated to anthocyanin concentration in the sample (J. Lee, Durst, & Wrolstad, 2005). Thus, a pH differential method was considered for the determination of anthocyanin content of strawberry juice (Meyers, Watkins, Pritts, & Liu, 2003). Potassium chloride was used as buffer 1 (0.025 M) at pH 1, and sodium acetate was utilized as buffer 2 (0.4 M) at pH 4.5 in this assay. 1 mL of SJ was transferred to a glass tube and followed by the addition of 9 mL of buffer 1. Likewise,

buffer 2 was added into another glass tube containing 1 mL of SJ. The tube contents were mixed thoroughly by using vortex. The absorbance values of the mixtures at pH 1 and 4.5 were then recorded by a spectrophotometer (HP 8452A diode array spectrophotometer, Agilent Technologies, Palo Alto, USA) at 510 and 700 nm against distilled water blank. The total anthocyanin content was calculated according to equation given below and expressed as mg pelargonidin-3-glucoside per L.

$$TAC = \frac{[(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}] \times MW \times df \times 1000}{\varepsilon \times L} \quad (4.6)$$

where A is the absorbance at 510 and 700 nm at given pH values, MW is the molecular weight of pelargonidin-3-glucoside (433.0 g/mol), df is the dilution factor, L is the path length in cm and ε is the molar extinction coefficient for pelargonidin-3-glucoside (22400 L/mol.cm).

4.3.3. Antioxidant Activity (RSA)

The free-radical scavenging activity (RSA) of SJ samples was evaluated by the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method of (Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2009) with slight modifications. Strawberry juice samples collected before and after treatments were centrifuged at 5000 rpm for 5 min at 4 °C. The supernatant was diluted 20 times by using MeOH-H_2O (3:2) solution. 3.9 mL of methanolic DPPH solution (0.025 g/L) was added to 0.01 mL of diluted supernatant. Then, the mixture was thoroughly shaken by a vortex at ambient temperature and kept in dark for 30 min. A control tube was prepared by using 0.01 mL of MeOH-H_2O (3:2) solution instead of juice samples. The absorbance of the samples was measured by HP 8452A diode array spectrophotometer (Agilent Technologies, Palo Alto, USA) at 515 nm. MeOH-H_2O (3:2) was used as blank to zero the spectrophotometer. For the accuracy of measurements, a calibration curve was constructed considering varying concentrations of Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Sigma Aldrich - 238813) up to 175 μM. The standard solutions were also treated in the same way as explained above. Antioxidant capacities of the samples were then calculated in percentage as given Equation (4.7).

$$\% \text{ inhibition} = \frac{(A_{DPPH} - A_{sample/std})}{A_{DPPH}} \times 100 \quad (4.7)$$

where A is the absorbance of the methanolic DPPH solution without sample and $A_{sample/std}$ is the absorbance of sample or standard Trolox solution after 30 min of incubation with methanolic DPPH solution.

4.4. Data Analysis

The data analysis was carried out by using Excel worksheet (Microsoft 2010) and Minitab 16 Statistical Software (Minitab Inc., UK), and GraphPad Prism 7.02 (GraphPad Software, Inc., La Jolla, CA, USA). The means of measured properties such as physicochemical and phytochemical attributes of strawberry juice samples subjected to different treatments were compared by Analysis of Variance (ANOVA) considering Tukey's comparison test at 95 % of confidence interval.

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were utilized in order to classify and distinguish the strawberry juice samples treated by different processing technologies with respect to physicochemical and phytochemical properties.

4.4.1. Principal Component Analysis

PCA is one of the unsupervised multivariate analysis technique by discriminating the available data without a prior knowledge of grouping (Kozak & Scaman, 2008). Theoretical basis of PCA relates to the transformation of original measurement variables into new variables called principal components (PCs). Each principal component is a linear combination of the original measurement variables (Banas et al., 2010). PCA reveals simplification of multivariate data by reducing the dimension of the data set and analysis of the structure of observations and the variables (Abdi & Williams, 2010; Kozak & Scaman, 2008). It can be used for the identification of combinations of variables having the largest contribution to the total variance in the data set. The PCs which explain a large amount of variation should be considered for the construction of two- or three- dimensional plots. In most of the cases, the first two

or three PCs provide the discrimination of the data by clustering the samples (Kozak & Scaman, 2008).

PCs enable the researchers to describe the multivariate information with considerable fewer variables. The original data matrix can be represented as (Brereton, 2003);

$$X = T.P + E \tag{4.8}$$

where

- X is the original data matrix;
- T refers to the scores having as many rows as the original data matrix;
- P are the loadings with the same amount of columns as the original data matrix;
- The number of columns in the T matrix is equal to the number of rows in the P matrix;
- E is the error matrix.

Equation 4.8 gives the constituents of PCA model. The model can be visualized considering the data, the scores, the loadings, and the residuals (Figure 4.4). The residuals (E) have the same structure as the original data (Bro & Smilde, 2014). PCA reduces the size of original variables to a number of significant principal components. Size reduction of variables reveals more manageable and easily interpretable dataset. The size of each component can be measured by eigenvalue. A simple definition of eigenvalue of a PC is the sum of squares of the scores. The proportion of the data that has been modeled by PCA is often approximately determined by cumulative percentage eigenvalue. The closer values to 100 % indicate more faithful model (Brereton, 2003).

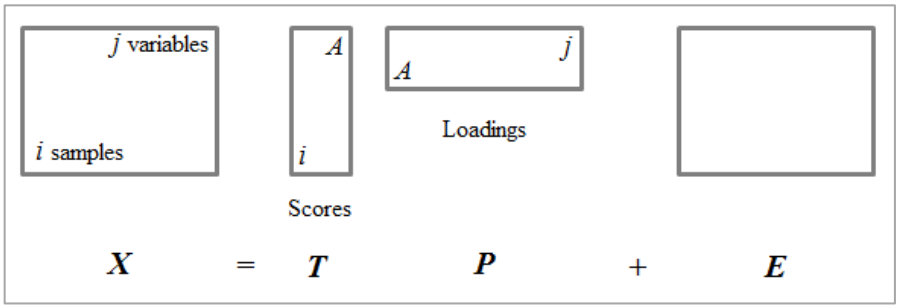


Figure 4.4. Visualization of PCA model
(Source: Brereton, 2003; Bro & Smilde, 2014)

4.4.2. Hierarchical Cluster Analysis

Cluster analysis identifies concentrated groups (i.e., clusters) among many samples while having no information about the groups. Therefore, it is called as one of the unsupervised techniques. In hierarchical cluster analysis (HCA), the samples agglomerate in a hierarchy and the optimal number of clusters forms; thereby hierarchical relations among samples are evaluated on tree-like plots (Brereton, 2003). The graphical representation of clusters is also called as dendrogram (Varmuza & Filzmoser, 2016). HCA is constructed considering the similarity or distances among observations (Huang, Guo, Qiu, & Chen, 2007). The samples are characterized based on the evaluation of the similarity between the samples by measuring the distances between the points in the measurement space. Dissimilar samples will be distant from each other, while the samples that are similar will lie close to one another (Banas et al., 2010).

Each sample has a relationship to the remaining samples. The similarity between each pair of samples can be defined in different ways. One of the most popular ways of determining how similar the samples are to each other is the correlation coefficient between samples. A correlation coefficient equal to 1 indicates that samples have identical characteristics. Another way of similarity determination is based on the distance between samples. Euclidean distance can be defined by the following equation.

$$d_{kl} = \sqrt{\sum_{j=1}^{j} (x_{kj} - x_{lj})^2} \tag{4.9}$$

where there are *j* measurements and x_{ij} is the *j*th measurement on sample i. d_{kl} shows the distance between sample k and sample l. The smaller is the value the more similar are the samples. Agglomerated clusters are gradually connected to each other forming a group in the dendrogram (Brereton, 2003).

CHAPTER 5

DETERMINATION OF EQUIVALENT CONDITIONS FOR MILD PASTEURIZATION OF STRAWBERRY JUICE

5.1. Introduction

Conventional thermal pasteurization has been proved to be effective on inactivation of microorganisms and enzymes (Chen & Wu, 1998). Processing parameters for thermal pasteurization of fruit juices are reported to be 90 °C for 1 min (Eagerman & Rouse, 1976), (95-98 °C for 10-30 s (Ringblom, 2004), 70 °C for a few seconds (FDA, 2004; Mazzotta, 2001). Due to the effects of thermal pasteurization towards undesirable changes in the product quality (Sandhu & Minhas, 2006), alternative processing technologies without direct heat have been emerged to facilitate microbial safety as well as preservation of nutrients and fresh-like properties (Zulueta, Barba, Esteve, & Frigola, 2013). Many studies related to the effect of nonthermal processing technologies on safety and quality of fruit juices can be found in literature. However, most of these studies have not been conducted considering equivalent inactivation of target microorganisms or enzymes. Therefore, it would not be possible to make a fair comparison among treatments. Comparison of different processes in terms of microbial safety, quality and shelf life of food products should be carried out under equivalent processing conditions that provide an equivalent effect, i.e. microbial inactivation (Vervoort et al., 2011).

In this study, equivalent processing approach was taken into consideration based on FDA's requirement of 5-log reduction of the pertinent microorganism (e.g. *E.coli* O157:H7) in the juice product (FDA, 2001). In order to find the processing conditions that satisfy this requirement, several parameters specific to high pressure processing (HPP), ultrasonication (US) and pulsed electric field (PEF) were studied by using a target microorganism. In this case, *E.coli* 11775 was used as a nonpathogenic surrogate of *E.coli* O157:H7 for strawberry juice (SJ). The conditions provided 5-log reduction of

E.coli 11775 in SJ subjected to each technology, i.e. high pressure processing (HPP), ultrasound (US), pulsed electrical field (PEF) and thermal processing, were selected as the equivalent processing conditions.

5.2. Material and Methods

A flow chart of the experimental design of this chapter is given in Figure 5.1.

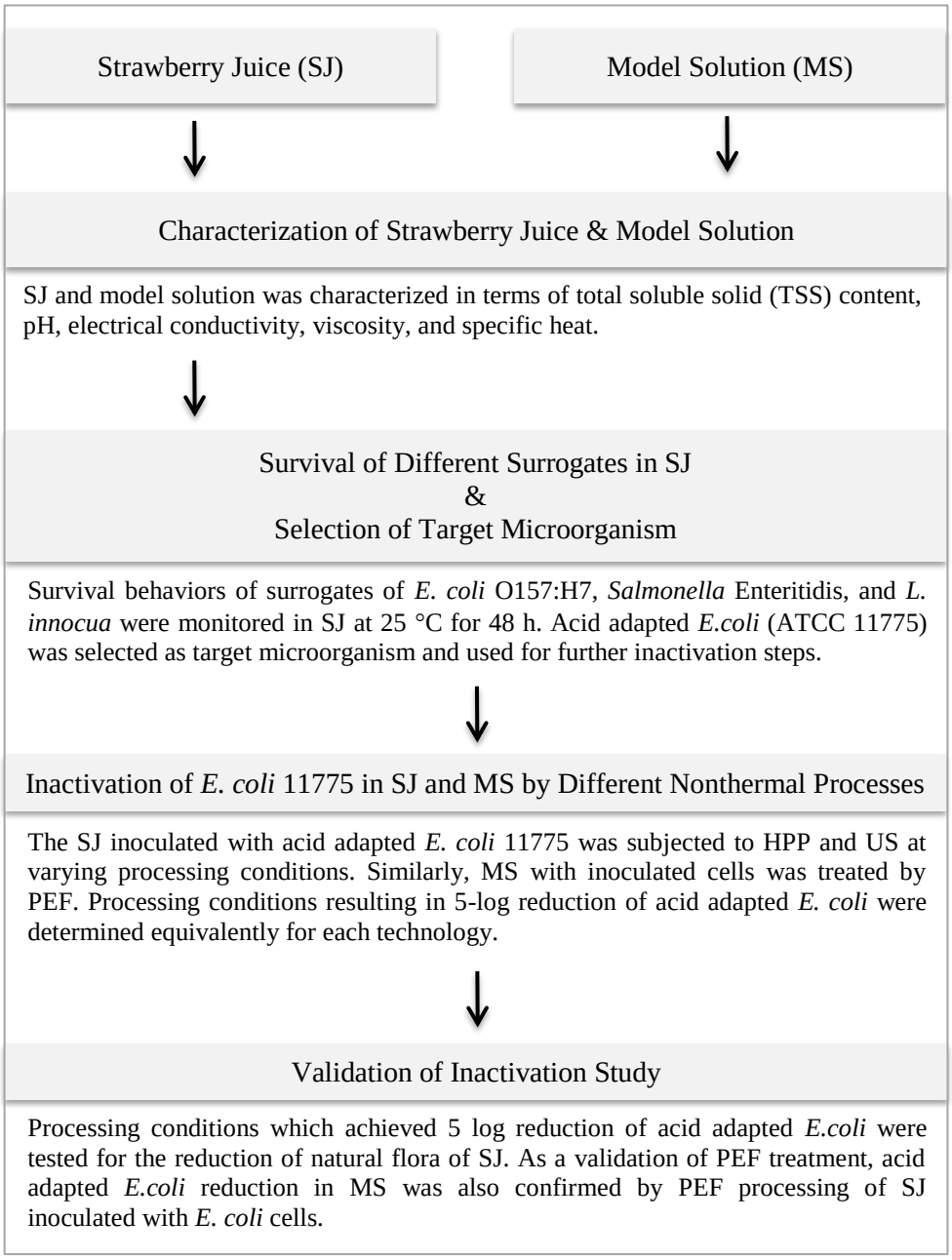


Figure 5.1. Flow chart of experimental design of Chapter 5

5.2.1. Preparation of Strawberry Juice and Model Solution

A pre-pasteurized strawberry juice prepared as described in Chapter 4 (section 4.1.2) was used for inoculation and inactivation of target non-pathogenic microorganism, i.e. *E. coli* ATCC 11775 by selected nonthermal and thermal technologies. With respect to PEF processing, a model buffer solution (MS) was used instead of strawberry juice because large amount of liquid (~6 L) was required to be passed through the PEF system. Thus, the model solution (MS) was developed by simulating strawberry juice in terms of electrical conductivity, pH, total soluble solid (TSS). The composition of the model solution was composed of citric acid (8 g/L), fructose (35 g/L), and glucose (35 g/L). pH of MS was adjusted to 3.4 by using 0.2 M Na_2HPO_4. Electrical conductivity of MS was set up that of strawberry juice (3.9 mS/cm) by the addition of 5 % NaCl solution.

5.2.2. Characterization of Strawberry Juice and Model Solution

Physicochemical Properties

pH of the samples were measured by a pH meter (FE20 FiveEasy, Mettler Toledo Columbus, OH, USA) at 22 ± 0.5 °C. Total soluble solid (TSS) content was measured by using digital hand-held pocket refractometer (PAL-BX/RI, Atago Co., Ltd., Tokyo, Japan). Finally, electrical conductivity of SJ and its model solution were measured by a benchtop pH – electrical conductivity meter (Orion 4 star, Thermo Scientific, USA). The probe of conductivity meter was immersed into the 15 mL of sample and the conductivity was recorded as mS/cm.

Thermal Properties

It was expected to have an increase in temperature of model solution and SJ was while running PEF system. The temperature increase in the treatment medium can be calculated considering the following equation:

$$\Delta T = \frac{Q}{\rho_f C_p} \tag{5.1}$$

where ρ_f and C_p are the density and specific heat of the fluid subjected to a processing technology, i.e. PEF processing (Zhang, Barbosa-Canovas, & Swanson, 1995). Similar specific heat values for SJ and model solution would lead to have similar heat transfer behavior of the liquids during processing. Thus, it was worth to analyze whether the energy taken by SJ and model solution were similar or not. Therefore, specific heat (J/g°C) of the samples was determined.

Specific heat of strawberry juice and model solution was measured by using a differential scanning calorimeter (DSC) Q2000 Series (TA Instruments, Inc., New Castle, DE, USA) with DSC manufacturer's software (TA Universal Analysis). The DSC was firstly calibrated with indium prior to use. About 5 to 8 mg of sample was sealed in aluminum pans. An empty aluminum pan was used as a reference. DSC runs were performed from 20–80°C at a heating rate of 10°C/min (Zainal, Rahman, Ariff, Saari, & Asbi, 2000). Based on the measured amount of energy (heat) absorbed a sample during a run, the heat flow data was analyzed to calculate the specific heat of strawberry juice samples and model solution.

Rheological Properties

Rheological measurements were conducted by a rheometer (MCR 300 rheometer, Anton Paar GmbH, Germany). The viscosity of strawberry juice and its model solution was measured under the controlled stress mode. A conic-end concentric cylinder geometry CC 27 (Paar Physica MCR 300, Anton Paar GmbH, Germany) was used in this study. The outer cylinder was stationary while the inner geometry was rotated. The viscosity was measured at varying temperatures between 20 and 60 °C (±0.5 °C). The temperature was controlled by a water bath (Viscotherm VT 10) connected to the rheometer. The change in shear stress and viscosity was monitored by changing the shear rate from 0.1 to 200 rpm during 5 min. Flow curves were constructed by using 12 data points obtained in the shear rate range of 11-200 rpm. Newtonian and Power Law models were applied for the rheological characterization of fluid materials.

Newtonian model is represented with the Equation 5.2:

$$\tau = \eta.\gamma \tag{5.2}$$

Power Law (Ostwald-de-Waele) model is given by the Equation 5.3:

$$\tau = k.\gamma^{n} \quad (5.3)$$

where τ is the shear stress (Pa), η is the viscosity, γ is the shear rate (s^{-1}), k is the consistency index, n is the flow behavior index.

5.2.3. Microbiological Studies

In this section, first of all, the survival of different surrogates in strawberry juice was studied to select the most resistant microorganism. Following this step, the SJ inoculated with this target microorganism was subjected to thermal and selected nonthermal technologies.

With respect to the survival and inactivation studies, a **pre-pasteurized SJ** was used in order to eliminate any error caused by the background microflora of the juice. For this purpose, the SJ was previously pasteurized at 71.7 °C for 15 s as explained in Chapter 4 (section 4.1.2). After pasteurization, the juice was cooled down to 25 °C by keeping in ice and used for the following steps.

5.2.3.1. Survival of Different Surrogates in SJ

Although *E.coli* O157:H7 has been reported to be the most resistant microorganism in juice products, on the first attempt, the growth behavior of different microorganisms in SJ were studied to determine survival potential of these bacterial contaminants. For this purpose, *Escherichia coli* (*E.coli*, ATCC 11775), *Enterococcus faecium* (*E. faecium*, ATCC 8459), *Listeria innocua* (*L. innocua*, ATCC 51742) were inoculated into SJ as surrogates of *E.coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes*, respectively.

E.coli (ATCC 11775) strain usually used as a surrogate of *E.coli* O157:H7 was gradually adapted to higher acidic conditions. In this respect, the cells were initially grown in nutrient broth (NB) (BD, Difco 234000) for 18 h at 37 °C. Then, 1 mL of this culture was transferred to another NB medium adjusted to pH 6.0 by citric acid, and

incubated for 18 h at 37 °C. In the same manner, the culture was then transferred to NB adjusted to pH 5.5, 5.0, 4.5, and finally 4.0 (Koutchma, Paris, & Patazca, 2007).

Frozen stock cultures of *E.coli* 11775 in glycerol stock solution (20 %, v/v) were activated and used for the further studies. In this regard, *E.coli* 11775 strain was firstly transferred to 100 mL nutrient broth from frozen stock culture and incubated at 37 °C for 18-24 hours. *E. faecium* was activated in Tryptic Soy Broth (TSB, Bacto 211825) by incubating at 37 °C 24 hours. Frozen stock culture of *L. innocua* was reactivated in TSB containing 0.6 % of yeast extract (YE, Difco 212750) at 37 °C for 5 h. The final medium was renamed as TSBYE.

100 mL of pre-pasteurized SJ was inoculated by target surrogates using 250 mL Erlenmeyer flasks. The final microbial population in the juice was adjusted to approximately 10^6 CFU/mL. The inoculated juice was then placed in a shaking water bath adjusted to 100 rpm. The survival behavior of each surrogate in the juice was monitored at 25 °C for 48 h.

Appropriate dilutions of SJ were prepared using 0.1 % pepton water. Samples containing *E. coli* cells were plated and counted on MacConkey Agar (BD, Difco 212123) by pour-plate method. The plates were incubated at 37 °C for 24 hours. Regarding *E. faecium*, the samples were plated on Tryptic Soy Agar (TSA, Difco 236950) using pour-plate method. *E. faecium* colonies were counted after incubation at 37 °C for 24 h. SJ samples with *L. innocua* cells, on the other hand, were plated on Tryptic Soy Agar (TSA) containing 0.6 % of yeast extract (TSAYE) using pour-plate method. The plates were counted for *L. innocua* colonies after incubation at 37 °C for 48 h.

5.2.3.2. Inoculation of SJ and Model Solution with Target Microorganism

Based on the survival behavior of surrogates (section 5.2.3.1) in SJ, acid adapted *E.coli* 11775 was selected as a target microorganism for SJ to be subjected to nonthermal and thermal processing technologies. In order to activate the stock culture, cells were grown in nutrient broth for 18 h at 37 °C. Once the *E.coli* cells reached to the early stationary phase, the culture was inoculated into pre-pasteurized strawberry juice

and exposed to selected processing technologies. The initial inoculation load of acid adapted *E.coli* was approximately 10^6 CFU/mL in the strawberry juice.

HPP was applied to sample pouches filled with SJ. For this purpose, 200 mL of SJ was placed in a beaker and inoculated with 1 mL of acid adapted *E.coli* (ATCC 11775) culture at 20 °C. The juice containing approximately 10^6 CFU/mL of *E.coli* cells was thoroughly mixed on a magnetic stirrer for 1 min. Then 20 mL of juice was aseptically transferred to plastic pouches (5×7 cm) (Nasco Whirl-Pak™, Fort Atkinson, WI). The pouches were then sealed by a hand operated sealer. Special attention was paid to avoid air bubbles inside the package. The contaminated juice samples in pouches were then subjected to high pressure processing conducted at different pressure and time variables.

The inactivation of acid adapted *E.coli* cells by sonication was performed at 25 °C, 40 °C and 55 °C. For this purpose, 400 mL of SJ was placed into a double wall sample container where the temperature of the juice was controlled by circulating cooling water through the walls. While running the sonicator, the temperature of the juice increased due to the energy transferred to the sample. Once the juice reached to the desired initial temperature (25, 40, or 55 °C) while running sonicator, *E. coli* cells were inoculated into SJ at a concentration of approximately 10^6 log CFU/mL and immediately mixed with magnetic stirrer bars.

Instead of strawberry juice, a model buffer solution was used for the inactivation of acid adapted *E. coli* 11775 cells by PEF processing. 6 L of buffer solution was inoculated with the target cells at a population of approximately 10^6 CFU/mL and exposed to PEF treatment based on central composite design. The processing conditions which provided at least 5-log reduction of acid adapted *E. coli* 11775 were then confirmed by PEF processing of SJ under the same operating conditions.

For conventional thermal pasteurization, 400 mL of pre-pasteurized strawberry juice (as described in Chapter 4.1.2) was put in a double walled sample unit connected to a water bath (Viscotherm VT 10, Paar Physica, Germany). The sample unit containing the juice was placed on a magnetic stirrer adjusted to 250 rpm. A magnetic stirrer bar was used in order to increase the heat transfer and homogenize the distribution of microorganisms inside the medium. The temperature of juice increased from 23.3 °C to 71.7 °C by circulating hot water (74 °C) around the sample unit. Once

the temperature reached to 71.7 °C, acid adapted *E. coli* cells were inoculated to the juice at a concentration of approximately 10^6 log CFU/mL.

5.2.4. Nonthermal and Thermal Processing of SJ Inoculated with Target Microorganism

5.2.4.1. High Pressure Processing of SJ

High pressure processing of SJ was carried out at Washington State University (WSU) by using a high hydrostatic pressure unit (Engineering Pressure Systems, Inc., Andover, MA, USA) with a cylindrical chamber vessel (0.1 m internal diameter, 0.25 m internal height). Figure 5.2 shows the HPP system used at Washington State University (WSU). The details of the system in schematic view are given in Figure 5.3.

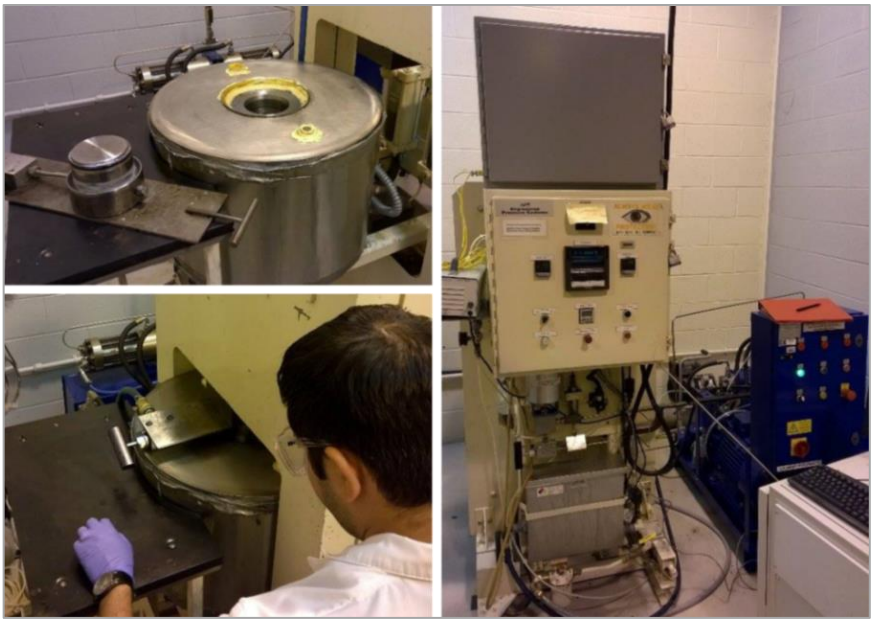


Figure 5.2. HPP system

A solution of 5% mobil hydrosol 78 in water was used as the pressurizing medium. The plastic pouches containing inoculated juice were placed in the chamber vessel. Then the chamber was filled with the pressurizing liquid. An electrohydraulic intensifier pump (Hochdruck-Systeme GmbH, AP 10-0670-1116, Sigless, Austria) was used to pressurize the vessel to the target pressure. SJ samples inoculated with acid

adapted *E.coli* 11775 were exposed to different levels of pressures (200, 250, 300, 350, 400 MPa) up to 2 min of treatment time. HPP treatments were conducted in triplicate. The temperature during the process was measured by a K-type thermocouple (Omega Engineering Inc., Stamford, CT). Temperature as well as pressure data were processed into profile charts through the use of a computer.

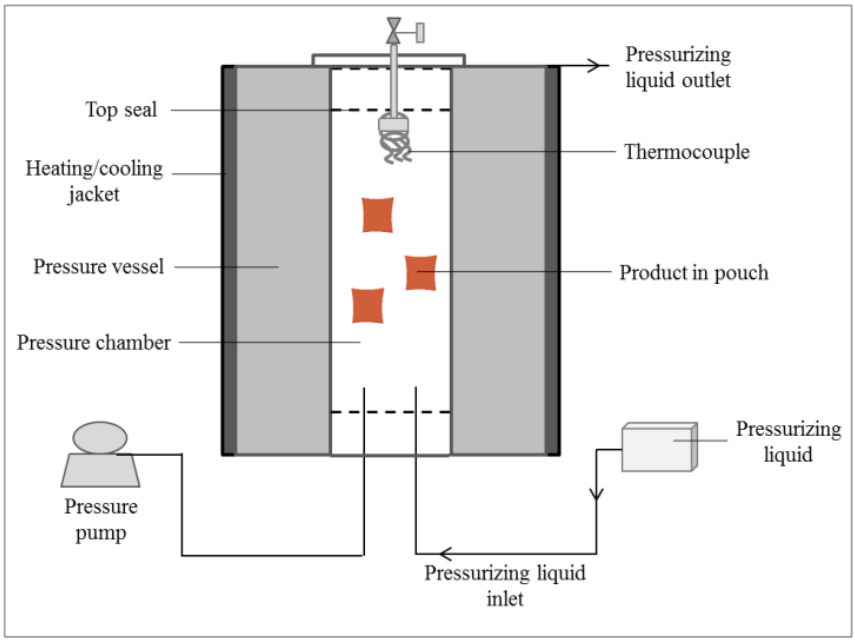


Figure 5.3. Schematic view of HPP system

5.2.4.2. Ultrasonication of SJ

Ultrasonication was applied by using an ultrasonic device (sonicator) (UP400S Hielscher USA Inc., Ringwood, NJ) equipped with a probe of 22 mm diameter (sonotrode H22) and a water bath (Viscotherm VT 10). Table 5.1 shows the technical specifications for the ultrasonicator. The maximum frequency and amplitude provided by the sonicator were 24 kHz and 120 μm, respectively.

Table 5.1. Technical specifications of ultrasonicator

Provider	Hielscher USA Inc., Ringwood, NJ, US
Model	UP400S
Power (W)	400
Frequency (kHz)	24
Maximum amplitude (μm)	120
Diameter of probe (mm)	22

Sonication was applied at three different temperatures (25, 40, 55 °C). Twenty five degree Celsius was chosen in order to reveal the effect of sonication itself. Sub-lethal and lethal temperature effects on the inactivation kinetics of target microorganism were studied at 45°C and 55°C. Four hundred mL of pre-pasteurized SJ was placed into the double wall sample container connected to a water bath. Two K-type probes were immersed into different locations of the SJ in order to monitor the temperature increase during treatment. While running sonicator, the temperature of the juice increases due to the energy transferred to the sample. Thus, the water circulating between the walls was adjusted to 4 °C, 15 °C, and 25 °C in order to keep the temperature of SJ at the desired corresponding temperatures of 25 °C, 40 °C, and 55°C, respectively. After running the sonicator, temperature of the juice sample increased to the treatment temperature by application of a certain come-up-time (CUT). Once the temperature of juice reached to the desired values (25, 40, 55 °C) after CUT, acid adapted *E.coli* cells were inoculated into the juce as described in section 5.2.3.2. The SJ inoculated with acid adapted *E.coli* 11775 were subjected to US up to 10 min. The treatment time was manually recorded by a chronometer. Sonication was applied up to 10 min considering 100 % of amplitude (120 μm) in continuous mode. Figure 5.4 represents schematic drawing of the ultrasonication system that was used in this study.

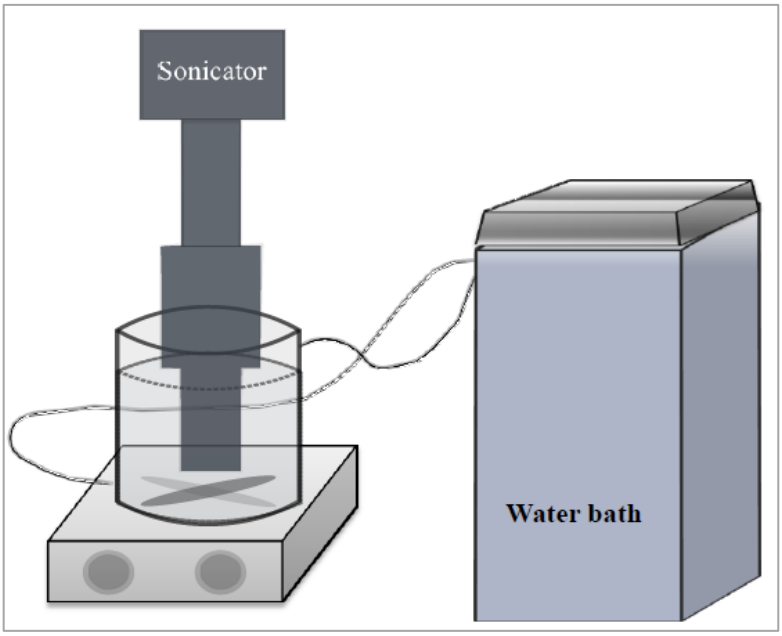


Figure 5.4. Schematic view of ultrasonicator

5.2.4.3. Pulsed Electric Field Processing of Model Solution

PEF processing was conducted at Washington State University (WSU). A pilot plant scale Powermod^{TM} pulsed electric field system manufactured by Diversified Technologies Inc. (DTI, Bedford, MA, USA) was used in this study (Figure 5.5). The whole treatment system is composed of sample containers, pump, pipes, treatment chamber containing electrodes, high voltage pulse generator, and cooling coil.

Figure 5.5. PEF system

The specifications of PEF system are given in Table 5.2. The system was equipped with two pairs of co-field treatment chambers having 0.50 cm in diameter and 0.65 cm in cell length. The flow chart of PEF processing is given in Figure 5.6. Since the PEF system requires high amount of sample (5-6 liter), a model solution (MS) was prepared to avoid the use of excessive amount of strawberry juice and reduce the cost of study. In order to determine the processing conditions that can achieve 5-log reduction of target microorganism, MS inoculated with acid adapted *E.coli* 11775 strain was subjected to PEF based on response surface methodology (RSM). In this respect, a face-centered central composite design (CCD) was constructed for inoculated model solution considering electrical field intensity (EFI) (25-35 kV/cm) and treatment time (5-27 μs) as independent parameters (Table 5.3). Independent variable levels were selected according to the operating conditions of PEF equipment. Factorial and axial points were

duplicated and three central points were added to check the reproducibility of the results.

Table 5.2. The specifications of PEF system

Average Output Power	25 kW maximum
Output Voltage	25 kV maximum
Output Pulse Current	300 A maximum
Pulse Width	1 μs – 10 μs
Pulse Repetition	3 kHz maximum
Operating Temperature	13ºC to 27ºC, relative humidity 80% non-condensing
Dual Treatment Chambers	2 pairs (4 treatment zones)
Gap Distance	0.8 cm
Electrode Diameter	0.6 cm
Conductivity (fluid)	0.8 ~ 5.0 mS / cm
Electric Field Strength	40 kV / cm maximum
Flow Rate	600 L/h (2.5 GPM) maximum

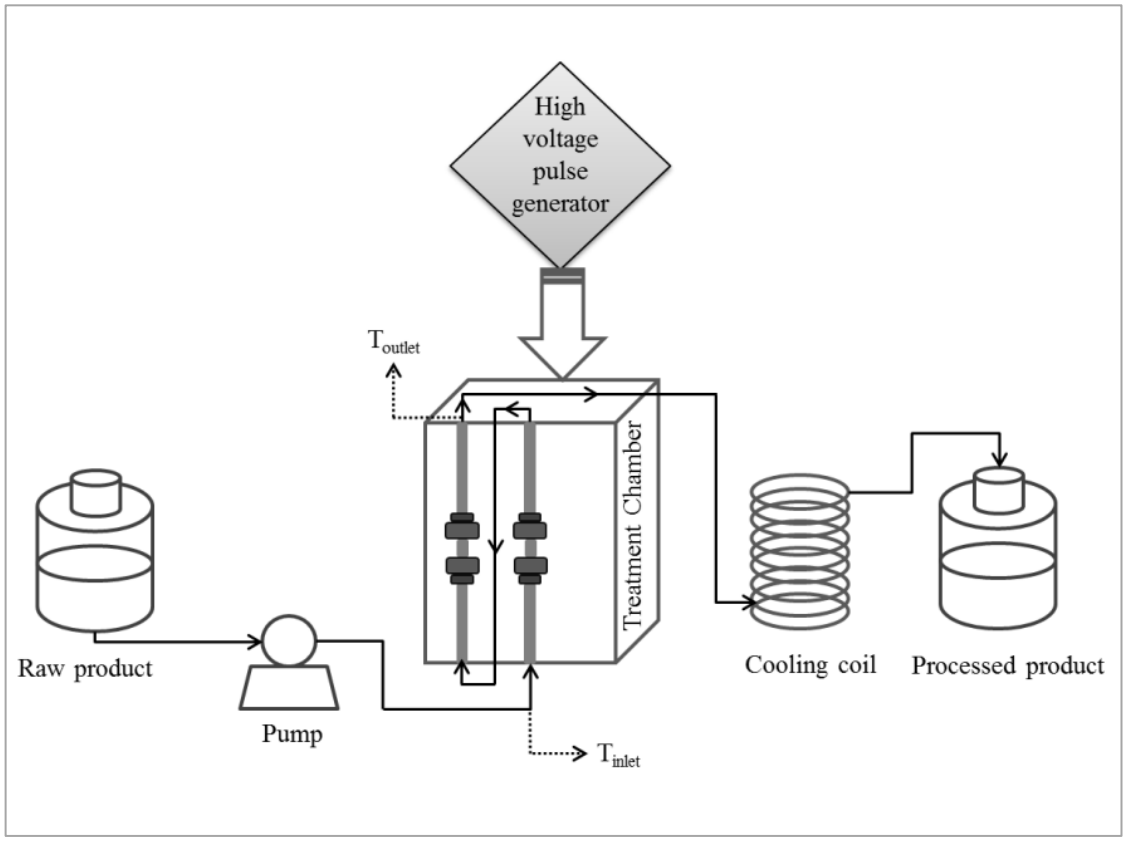


Figure 5.6. Flow chart of PEF processing

Table 5.3. Factors and levels of CCD matrix

Factor	Low (-1)	Center (0)	High (+1)
Electric field intensity (EFI) (kV/cm)	25	30	35
Treatment time (μs)	5	16	27

Approximately 6 L of model solution inoculated with acid adapted *E.coli* was passed through the system at a flow rate of 350 mL/min. The temperature of model solution was measured at the inlet and outlet of treatment chamber by two thermocouples (K-type, Omega Engineering, Inc., Stamford, CT, USA) (Figure 5.6). Electric field intensity (EFI), frequency, and pulse width was adjusted by the control panel of the PEF system (Figure 5.7). An oscilloscope (Hewlett Packard 54520A) was used to monitor the shape and width of monopolar electric pulses (Figure 5.8).

Figure 5.7. Control panel of PEF equipment

Figure 5.8. Monitoring the pulses by oscilloscope

In this study, a constant pulse width (2 μs) was applied in monopolar square waveform. After cooling the juice by a cooling coil, the treated samples were collected

and used for enumeration of *E. coli* 11775 cells. The findings of this study carried out with model solution were then confirmed by using strawberry juice. Likewise, the strawberry juice was inoculated with acid adapted *E.coli* 11775 and exposed to the same PEF conditions which provided at least 5-log reduction in the model solution.

5.2.4.4. Thermal Processing of SJ

Thermal processing of SJ inoculated with acid adapted *E. coli* cells was conducted by using the same pasteurization unit given in section 4.1.2. Briefly, the juice was inoculated with acid adapted *E. coli* cells once the temperature of juice reached to 71.7 °C. In order to monitor the inactivation of inoculated cells, sampling had been done at different time intervals during 15 s of processing time.

5.2.4.5. Validation of Processing Conditions

Freshly squeezed SJ was exposed to HPP, US, PEF and thermal processing under the conditions that were obtained from the previous inactivation studies. The equivalent processing conditions of these treatments were confirmed by examining their effect on the natural microbial flora of SJ, i.e., total mesophilic aerobic count (TMAC) and yeast-mold (YM) count. For this purpose, the freshly squeezed strawberry juice was kept at 4 °C for 5 days in order to increase its microbial content. Then, the juice with increased natural microbial flora was exposed to HPP, US, PEF, and thermal pasteurization under the conditions that yielded at least 5-log reduction of acid adapted *E.coli* 11775 cells. The reduction in the number of TMAC and YM count of SJ was evaluated.

5.2.4.6. Microbial Growth and Enumeration of Microorganisms

After subjecting the SJ inoculated with acid adapted *E. coli* cells to the nonthermal and thermal treatments, the samples plated on MacConkey Agar (BD, Difco 212123) by pour-plate method. For this purpose, appropriate dilutions were prepared using 0.1 % pepton water. The *E. coli* cells were enumerated after incubation at 37 °C

for 24 hours. On the other hand, total mesophilic aerobic count of treated and untreated SJ samples were enumerated by plating on Plate Count Agar (PCA). The plates were then incubated at 37 °C for 48 h, and the colonies were counted. Additionally, the yeast-mold load of treated and untreated SJ was plated on Potato Dextrose Agar (PDA) acidified with tartaric acid (10 %). The plates were incubated at 25 °C for 5 days and then counted for the yeasts and molds.

5.2.5. Data Analysis

The data analysis was carried out by using Excel worksheet (Microsoft 2010) and Minitab 16 Statistical Software (Minitab Inc., UK), and GraphPad Prism 7.02 (GraphPad Software, Inc., La Jolla, CA, USA).

The data related to the characterization of strawberry juice and its model solution was conducted considering t-test. The means of measured properties such as TSS, pH, electrical conductivity, viscosity, and specific heat of SJ and MS were compared considering 95 % of confidence interval. The null hypothesis was that there would be no difference between SJ and MS in terms measured properties. Significance was defined for *p* values less than 0.05. Regarding rheological properties of SJ and MS, Newtonian and Power Law models were applied. RMSE and R^2 values of both models were compared for the evaluation of flow characteristics of SJ and its model solution.

Regarding the survival study of different surrogates in strawberry juice, Analysis of Variance (ANOVA) was applied to compare the resistances of the microorganism in SJ. The data for inactivation of the acid adapted *E.coli* by different processes was evaluated by monitoring the inactivation kinetics by each technology. The equivalent conditions which provided 5-log reduction of acid adapted *E.coli* cells in strawberry juice were determined for each technology. TMAC and YM counts of SJ subjected to different treatments were compared by ANOVA. The data related to the microbiological studies were given as mean ± standard deviation.

5.3. Results and Discussion

5.3.1. Characterization of Strawberry Juice and Model Solution

Physicochemical Properties

Special attention needs to be paid while preparing the model solution because it should simulate significant properties of the real strawberry juice. It is known that microbial inactivation by PEF technology can be highly influenced by the electrical conductivity of the medium since any change in the conductivity affects the pulse energy delivered to the product (Wouters, Alvarez, & Raso, 2001). Thus, electrical conductivity is a critical parameter for PEF processing; it has to be taken into consideration while preparing model solution. The properties of model solution were closely adjusted to real SJ (Table 5.4). The electrical conductivities of freshly squeezed SJ and MS were 3.96 and 3.90 mS/cm, respectively.

pH of model solution was also adjusted to that of strawberry juice. As given in Table 5.4, pH of SJ and MS were 3.4 and 3.39, respectively. These results were in agreement with the others. Tiwari et al. (2008) applied sonication to strawberry juice with a pH value of 3.14 ± 0.05 (Tiwari, O'Donnell, Patras, & Cullen, 2008). Accordingly, Tiwari et al. (2009b) characterized the physicochemical properties of strawberry juice before ozone processing. The authors used strawberry juice having a pH value of 3.11 ± 0.03 (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009b). Bhat and Stamminger (2015) reported the pH value as 3.18 ± 0.02 for the strawberry juice subjected to UV-C light treatment (Bhat & Stamminger, 2015).

Table 5.4. Physicochemical properties of strawberry juice and model solution

Properties	**Strawberry juice**	**Model solution**
Electrical conductivity (mS/cm)	3.96	3.90
pH	3.4	3.39
Total soluble solids (°Brix)	8.05	7.9

Besides, total soluble solid content (°Brix) of model solution was also successively adjusted to the similar levels of strawberry juice as shown in Table 5.4. TSS of SJ and MS were 8.05 and 7.9 °Brix, respectively. The °Brix value of strawberry juice used for ozonation by Tiwari et al. was reported to be 9.82 ± 0.41 (Tiwari et al.,

2009b). In another study, a relatively higher content of total soluble solids, i.e. 11.6 °Brix for strawberry juice having a pH value of 3.7 (Duan & Zhao, 2009). These discrepant results could be aroused from differences in varieties, chemical composition, as well as fruit juice preparation operations such as homogenization, centrifugation, filtration, clarification (Chatterjee, Chatterjee, Chatterjee, & Guha, 2004; Falguera, Pagan, & Ibarz, 2011b; Schols, Intveld, Vandeelen, & Voragen, 1991).

Thermal Properties

Specific heat capacity of liquids changes as a function of temperature. A temperature increase during PEF processing was expected due to the dissipation of electrical energy in the form of thermal energy (Jaeger, Meneses, Moritz, & Knorr, 2010). Therefore, specific heat value of MS was expected to be close to that SJ in order to have similar heat transfer characteristics. Figure 5.9 shows the specific heat versus temperature graph.

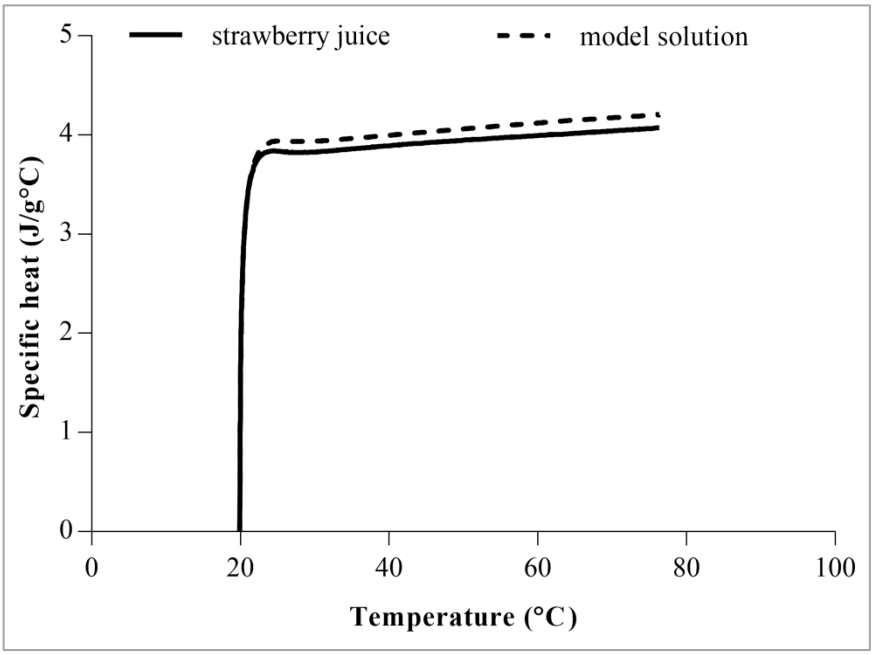


Figure 5.9. Change in specific heat capacity (Cp) of SJ and MS as a function of temperature

Two-sample t-test with 95 % of confidence interval was applied for specific heat data of SJ and MS. Specific heat of MS was not significantly different than SJ considering the temperature range of 22 °C - 75 °C. *p* values from t-test are given in Table 5.5. *p* value higher than 0.05 shows non-significant difference between samples.

Therefore, it can be concluded that the specific heat of model solution and strawberry juice was similar. This means that when model solution was treated by PEF, theoretically the heat transfer behavior of MS would be similar to SJ. Specific heat capacity values measured for SJ and MS were 3.71 ± 0.41 and 3.75 ± 0.16 J/(g°C), respectively. As the temperature increased to 75 °C, specific heat was also increased up to 4.07 ± 0.36 and 4.20 ± 0.01 for SJ and MS, respectively. The values found in our study were higher than those found by Dubrovic et al. (2011) and Tiwari et al. (2009a). Dubrovic et al. (2011) and Tiwari et al. (2009a) reported the specific heat capacity of strawberry juice as 3.368 kJ/(kg·°C) (Dubrovic, Herceg, Jambrak, Badanjak, & Dragovic-Uzelac, 2011) and 3.31 kJ/(kg °C) (Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009a). The differences might be because of the varietal differences of fruits and specific heat measurement procedures used in different studies.

Table 5.5. Specific heat capacity (Cp) of strawberry juice and its model solution at different temperatures

Temperature (°C)	**C_p (J/g°C)**		***p*-value**
	Strawberry Juice	**Model Solution**	
22	3.71 ± 0.41	3.75 ± 0.16	0.918
25	3.83 ± 0.39	3.94 ± 0.10	0.779
30	3.83 ± 0.39	3.94 ± 0.09	0.759
35	3.86 ± 0.38	3.96 ± 0.09	0.770
40	3.89 ± 0.38	4.00 ± 0.08	0.766
45	3.92 ± 0.38	4.03 ± 0.07	0.759
50	3.95 ± 0.38	4.06 ± 0.06	0.746
55	3.97 ± 0.37	4.09 ± 0.05	0.731
60	3.99 ± 0.37	4.12 ± 0.04	0.715
65	4.02 ± 0.37	4.15 ± 0.02	0.703
70	4.04 ± 0.36	4.17 ± 0.02	0.696
75	4.07 ± 0.36	4.20 ± 0.01	0.696

Rheological Properties

The flow behavior of model solution and strawberry juice was evaluated considering Newtonian and Power Law models (Figure 5.10). Shear stress (τ) and shear rate (ϒ) values were plotted for Newtonian model. The slope of the graph at different temperature was calculated as viscosity (mPa.s). Regarding the power law model, log (τ) and log (ϒ) was plotted, slope and intercept values were used as flow behavior index (n) and consistency index (K), respectively. RMSE and R^2 values were calculated. The

model parameters of Newtonian and Power Law models for SJ and MS were depicted İN Table 5.6 and Table 5.7. According to Newtonian model, as the temperature increased the viscosity decreased as expected. The flow behavior of MS and SJ showed similar trend and perfectly described by Newtonian model with lower RMSE and higher R^2 values. The viscosity could increase with the increasing concentration of soluble content (Giner, Ibarz, Garza, & XhianQuan, 1996). Nindo et al. (2005) studied even more concentrated berry juices, i.e. blueberry and raspberry, with soluble content up to 65 °Brix at temperatures varied from 20 and 60 °C. Accordingly, the authors stated that the flow behaviors of the two type of berry juices were predominantly Newtonian (Nindo, Tang, Powers, & Singh, 2005).

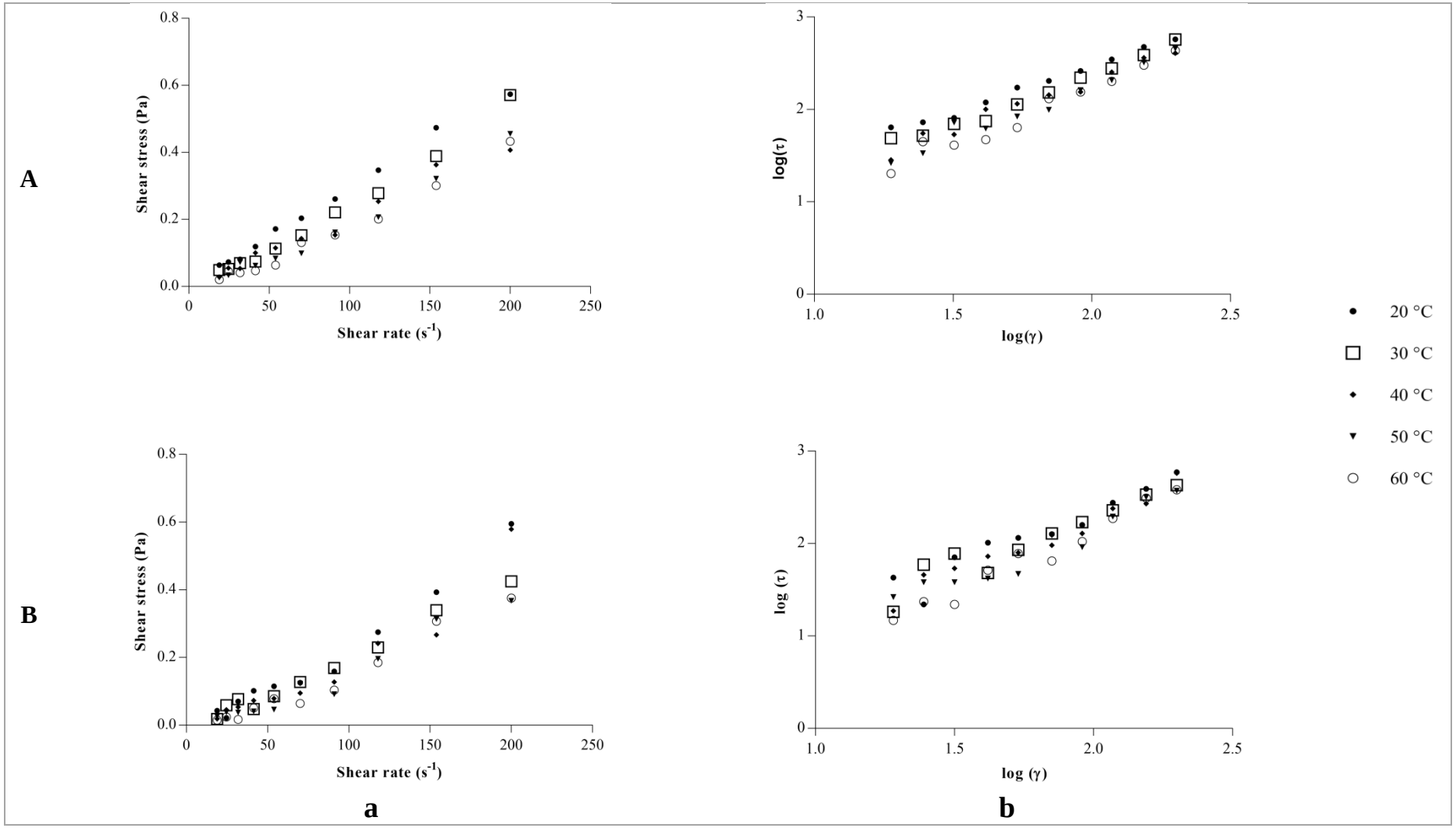


Figure 5.10. Shear stress (τ) versus shear rate (γ) plot for SJ (A) and MS (B) at different temperatures (a: Newtonian, b: Power Law Model)

Table 5.6. The parameters of Newtonian and Power Law Models describing the flow behavior of strawberry juice

Temperature (°C)	Newtonian model			Power law model			
	Slope (viscosity) mPa.s	RMSE	R^2	Slope (flow behavior index, n)	Intercept (Consistency index, k)	RMSE	R^2
20	2.53	0.01	0.9892	0.95	1.15	0.10	0.986
30	2.32	0.01	0.9911	1.13	0.20	0.13	0.9833
40	2.09	0.02	0.9727	1.17	-0.12	0.14	0.9824
50	1.96	0.03	0.9550	1.20	-0.38	0.21	0.9657
60	1.86	0.03	0.9451	1.14	-0.20	0.21	0.9611

Table 5.7. Flow behavior properties of model solution

Temperature (°C)	Newtonian model			Power law model			
	Slope (viscosity mPa.s)	RMSE	R^2	Slope (flow behavior index, n)	Intercept (Consistency index, k)	RMSE	R^2
20	2.19	0.03	0.9519	1.20	-0.25	0.29	0.9323
30	1.91	0.02	0.9616	1.06	0.22	0.24	0.9434
40	1.90	0.02	0.9647	1.34	-1.04	0.17	0.9818
50	1.74	0.04	0.8923	1.24	-0.60	0.21	0.9648
60	1.61	0.02	0.9602	1.24	-0.60	0.32	0.9262

5.3.2. Survival of Surrogates in Strawberry Juice

Although pathogenic microorganisms do not grow in fruit juices due to the low pH, they can survive or adapt to the acidic medium (Mazzotta, 2001). Survival attitudes of different surrogates, i.e. *L. innocua, E. faecium, and E. coli* 11775, in strawberry juice during 48 hours of incubation at 25 °C are shown in Figure 5.11. The reduction in the number of each surrogate bacteria was monitored in order to determine the most resistant microorganism in the juice. During 12 hours, microorganisms were not significantly affected from pH of strawberry juice (pH 3.4). The rate of decrease in the number of cells increased after 12 hours. At the end of 48 hours, the counts of *L. innocua, E. faecium, E. coli,* and acid adapted *E. coli* were reduced by 2.06 ± 0.45, 2.72 ± 0.03, 1.93 ± 0.27, and 1.54 ± 0.04 log CFU/mL, respectively.

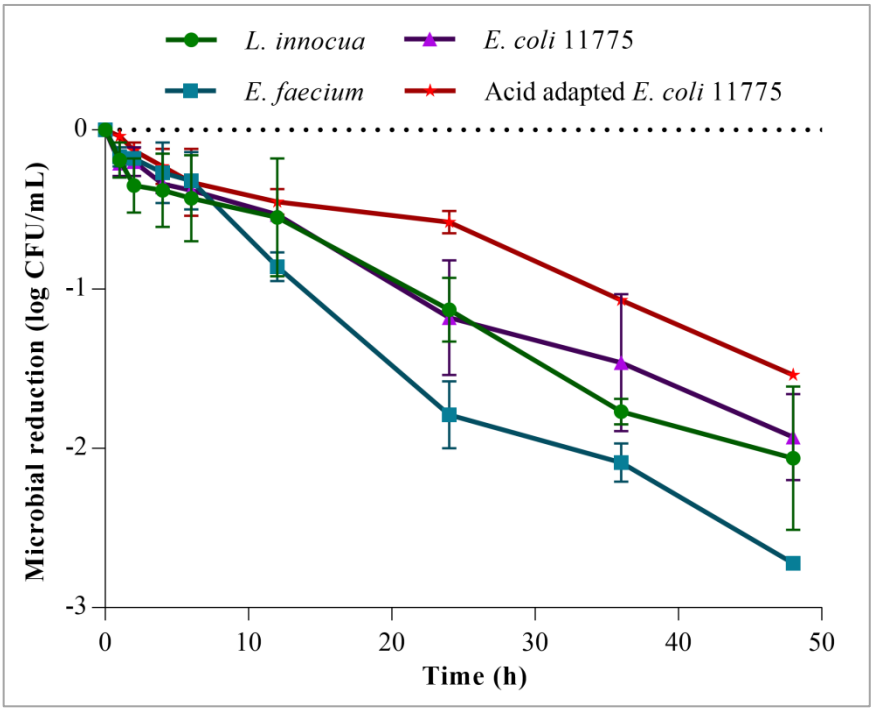


Figure 5.11. Logarithmic reduction of surrogates in SJ

Table 5.8 shows the logarithmic reduction of *L. innocua, E. faecium, E. coli* 11775 and acid adapted *E. coli* 11775 inoculated in strawberry juice. ANOVA with Tukey comparison test showed no significant difference in the logarithmic reductions of these microorganisms during 12 hours. At 24, 36 and 48th hours, acid adapted *E.coli* 11775 cells showed the least reduction. In other words, they showed the highest

resistance to the acidic medium of SJ having pH value of 3.4. Han and Linton (2004) studied survival and growth behavior of *E. coli* O157:H7 in SJ and acidified media adjusted to different pH values. For this purpose, the authors inoculated the juice and the media with approximately 6.7 log CFU/mL of *E. coli* O157:H7 and subjected to incubation at 4 °C and 37 °C for 3 days. They claimed that *E. coli* cells survived well at pH values varying from 3.4 to 6.8 at 4 °C. The number of injured cells increased with decreasing pH values and increasing incubation time (Han & Linton, 2004). This finding is in accordance with the results of this study. Similarly, Han and Linton (2004) reported that *E. coli* O157:H7 was inactivated at pH values lower than 3.6 at 37 °C whereas the cells were able to grow at pH 4.7 (Han & Linton, 2004). Moreover, *E. coli* O157:H7 isolated from a patient associated with the apple cider-related outbreak was studied in unpasteurized apple ciders having pH values varying from 3.6 to 4.0. It was reported that *E. coli* O157:H7 cells survived from 10 to 31 days or 2 to 3 days at 8 °C or 25 °C, respectively, depending on the lot of cider (Zhao, Doyle, & Besser, 1993).

Table 5.8. Logarithmic reduction of surrogates in SJ during 48 h (log CFU/mL)

Time (h)	***L. innocua***	***E. faecium***	***E. coli***	**Acid adapted *E. coli***
0	0.00	0.00	0.00	0.00
1	0.19 ± 0.11a	0.17 ± 0.06a	0.21 ± 0.08a	0.04 ± 0.11a
2	0.35 ± 0.17a	0.18 ± 0.03a	0.20 ± 0.09a	0.13 ± 0.05a
4	0.38 ± 0.23a	0.27 ± 0.19a	0.34 ± 0.06a	0.23 ± 0.11a
6	0.43 ± 0.27a	0.32 ± 0.18a	0.38 ± 0.02a	0.33 ± 0.21a
12	0.55 ± 0.37a	0.86 ± 0.09a	0.53 ± 0.04a	0.45 ± 0.08a
24	1.13 ± 0.20b	1.79 ± 0.21a	1.18 ± 0.36b	0.58 ± 0.07c
36	1.77 ± 0.08ab	2.09 ± 0.12a	1.46 ± 0.43ab	1.07 ± 0.03b
48	2.06 ± 0.45ab	2.72 ± 0.03a	1.93 ± 0.27ab	1.54 ± 0.04b

Results were expressed as mean ± std. Different letters in the same row shows the significant differences among surrogates at certain times.

Raybaudi-Massilia et al. (2009) indicated *E. coli* O157:H7 as a vehicle of foodborne illnesses due to the outbreaks in apple cider and apple juice. Salmonella outbreaks, on the other hand, have been observed especially associated with orange juice (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martin-Belloso, 2009). Thus, juice processors can either choose *E. coli* O157:H7 or *Salmonella* in order to determine the lethal effect of pasteurization treatment (Mazzotta, 2001). National

Advisory Committee on Microbiological Criteria for Foods recommended *E. coli* O157:H7 or *L. monocytogenes* to be used as target organisms in the absence of known specific pathogen-product associations (FDA, 1998). Acid adaptation of such pathogenic microorganism should be also considered since adaptation to acidic environment increased the heat resistance of *E. coli* O157:H7 and *L. monocytogenes* in apple, orange and white grape juice (Mazzotta, 2001). In this study, acid adapted *E.coli* 11775 cells showed higher resistance to pH of SJ than other surrogates. Therefore acid adapted *E.coli* 11775 was selected as a target microorganism for the inactivation studies to be carried out using different nonthermal technologies.

Most of the incidents of foodborne illness reported for the fruit juices were related to the unpasteurized juices (FDA, 1998). As a matter of fact, a pasteurization process, which is effective on the most resistant pathogen likely to occur in the juice, can also achieve the control of other microorganisms (Mazzotta, 2001). In conclusion, this study led us to identify the most resistant surrogate that can most likely to survive during the first two days of freshly squeezed strawberry juice. Thus, inactivation of this target microorganism, i.e. acid adapted *E.coli*, by different processing technologies can be considered for the next sections in order to guarantee the microbial safety of SJ prior to consumption.

5.3.3. Inactivation of Acid Adapted *E.coli* in SJ by HPP

The effect of HPP on acid adapted *E.coli* 11775 in SJ at varying pressures and processing times is depicted in Figure 5.12. HPP applied at 200 and 250 MPa for 120 s were able to reduce the number of acid adapted *E. coli* in SJ by 3.52 and 4.02 log CFU/mL, respectively. On the other hand, HPP treatments at higher pressures resulted in at least 5-log reductions in the number of acid adapted *E.coli* 11775 in SJ (Table 5.9). The treatment of SJ at 300 MPa for 45 s and 60 s resulted in greater than 5-log reduction of *E. coli*. Similarly, the treatments at 350 MPa for 30 s and 400 MPa for 15 s achieved 5.85 and 6.01 log CFU/mL reductions of *E. coli* in SJ, respectively (Table 5.9).

There are several studies investigating the inactivation of different *E. coli* strains in either buffer systems or juice (Garcia-Graells, Hauben, & Michiels, 1998; Van Opstal, Vanmuysen, Wuytack, Masschalck, & Michiels, 2005). The findings of this

study were in good agreement with these studies. For example, Tahiri et al. (2006) applied dynamic high pressure for the inactivation of *E. coli* O157:H7 (ATCC 35150) in orange juice. The authors achieved 5-log reduction and complete inactivation of *E. coli* cells in orange juice at 200 MPa and 25 °C after 3 and 5 passes, respectively (Tahiri, Makhlouf, Paquin, & Fliss, 2006). Application of HPP at 300 MPa for 5 min initially caused a smaller reduction of *E. coli* (NCFB 1989) between 1.7 and 3 log cycles in orange, tomato, and apple juice. However, no survival cells were observed for any type of fruit juice after a subsequent storage at 5 °C for 24 h (Jordan, Pascual, Bracey, & Mackey, 2001).

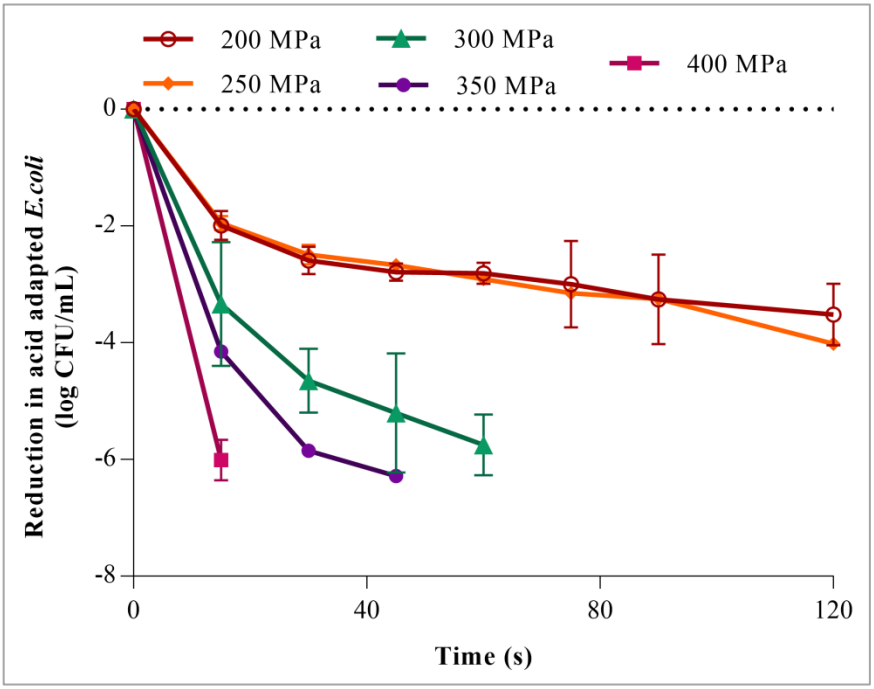


Figure 5.12. Inactivation of acid adapted *E. coli* 11775 in SJ by HPP

Teo et al. (2001) studied HPP inactivation of *E. coli* O157:H7 in unpasteurized fruit juices by using a cocktail of three different strains (SEA13B88, ATCC 43895, and 932). The viability losses in the *E.coli* cocktail was reported to be 0.41, 2.16, 6.40, and 8.34 log for apple, orange, grapefruit, and carrot juice, respectively, after subjecting juices to 615 MPa for 1 min (Teo, Ravishankar, & Sizer, 2001). Previous studies showed that increasing the pH of the medium resulted in the more pressure-resistant microorganisms (Garcia-Graells et al., 1998). Teo el al. (2001) attributed the reason of this situation to the recovery of pressure-injured *E. coli* cells in medium having low pH values, i.e. apple juice. But this subject needs a further confirmation. Ramaswamy et al.

(2003) studied HPP destruction of *E. coli* 29055 in apple juice considering varying pressure and time parameters. The authors used different types of media, i.e. violet-red bile agar (VRBA) and brain-heat infusion agar (BHIA), for enumeration in order to evaluate both healthy and injured cells, respectively. It was reported that HPP at 350 MPa was able to achieve complete inactivation of *E. coli* population (10^8 CFU/mL) at 1, 2, and 3 min of holding times. However, this finding was obtained when the juice samples were plated on VRBA which allowed only healthy cells to be counted. The authors also enumerated injured cells using BHIA media. Therefore, they emphasized not to underestimate the presence of injured cells due to the their possible recovery in time (Ramaswamy, Riahi, & Idziak, 2003). Additionally, subsequent death during storage should be also taken into consideration (Jordan et al., 2001).

Table 5.9. Reduction of acid adapted *E.coli* 11775 by HPP (log CFU/mL)

Time	**Pressure (MPa)**				
(s)	**200**	**250**	**300**	**350**	**400**
0	0.00	0.00	0.00	0.00	0.00
15	1.99 ± 0.25	1.95 ± 0.12	3.34 ± 1.06	4.15 ± 0.04	6.01 ± 0.35
30	2.59 ± 0.23	2.49 ± 0.17	4.65 ± 0.55	5.85 ± 0.05	
45	2.79 ± 0.15	2.67 ± 0.11	5.20 ± 1.02	6.28 ± 0.11	
60	2.81 ± 0.18	2.91 ± 0.04	**5.75 ± 0.52**		
75	3.00 ± 0.74	3.15 ± 0.07			
90	3.26 ± 0.77	3.25 ± 0.01			
120	3.52 ± 0.53	4.02 ± 0.07			

The temperature and pressure profiles of HPP treatment at 300 MPa are shown in Figure 5.13. The initial temperature (t_0) of the pressurizing liquid inside the treatment chamber was 18.33 ± 1.04 °C. Due to the compression heating; the temperature reached to initial holding temperature (t_1) of 24.70 ± 1.47 °C following 0.5 min come-up-time (CUT). The pressure CUT and depressurization times were shorter compared to the holding time. During holding time of 1 min under 300 MPa, the temperature was slightly elevated up to 26.13 ± 1.10 °C. Then, decompression took place during 0.4 min and the temperature reduced to its initial level along with the decreasing pressure.

Depending on the type of food product and target microorganism of concern, commercial exposure times can vary from millisecond pulse (by oscillating pumps) to a holding time of 20 min. From economical point of view, practical exposure times may be limited to less than 20 min (FDA, 2014). In this study, the holding time at elevated

pressure was 1 min. Similarly, Balasubramaniam et al. (2015), on the other hand, recommended a processing holding time of less than 10 min in order to develop a commercially viable process (Balasubramaniam, Martiinez-Monteagudo, & Gupta, 2015). Likewise, many studies related to the HPP of fruit juices applied processing times up to 5 min in order to achieve 5-log reduction of target microorganism (Guerrero-Beltran, Barbosa-Canovas, & Welti-Chanes, 2011; Ramaswamy et al., 2003).

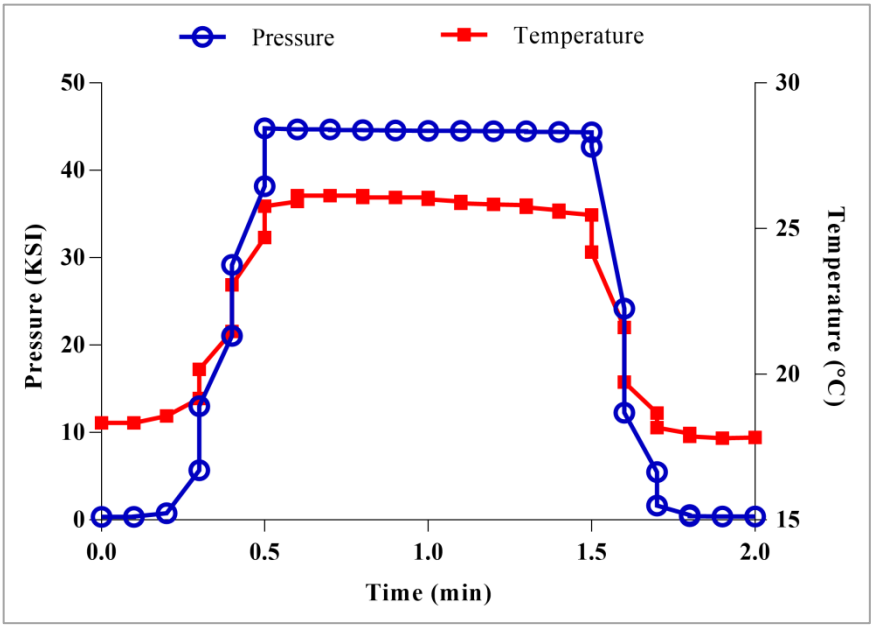


Figure 5.13. Temperature and pressure profiles of HPP treatment at 300 MPa

In conclusion, 300 MPa and 60 s were selected as the final HPP conditions for pasteurization of SJ on the basis of FDA's 5-log reduction requirement in the pertinent target microorganism for strawberry juice.

5.3.4. Inactivation of Acid Adapted *E.coli* in SJ by US

Figure 5.14 represents the inactivation kinetics of acid adapted *E.coli* cells in SJ exposed to US at 25, 40, and 55 °C. Sonication at sub-lethal temperatures, i.e. 25 °C and 40 °C, was monitored for 10 minutes. After exposing the inoculated strawberry juice to sonication at 25 °C and 40 °C, the logarithmic reductions of acid adapted *E.coli* were found as 1.65 ± 0.07 log CFU/mL and 3.58 ± 0.53 log CFU/mL, respectively. This finding indicated that sonication at sub-lethal temperatures was not sufficient to satisfy FDA's 5-log reduction requirement for the inactivation of acid adapted *E. coli* 11775 in

SJ. On the other hand, the number of acid adapted *E.coli* cells was reduced by 5.69 ± 0.61 log CFU/mL when the juice was sonicated at 55 °C for 3 min. Sonication in combination with mild heating, i.e. thermosonication, successively increased the reduction in the number of acid adapted *E. coli* 11775 cells in SJ. Hence, the combination of mild heating with sonication provided an advantage for complete lethality.

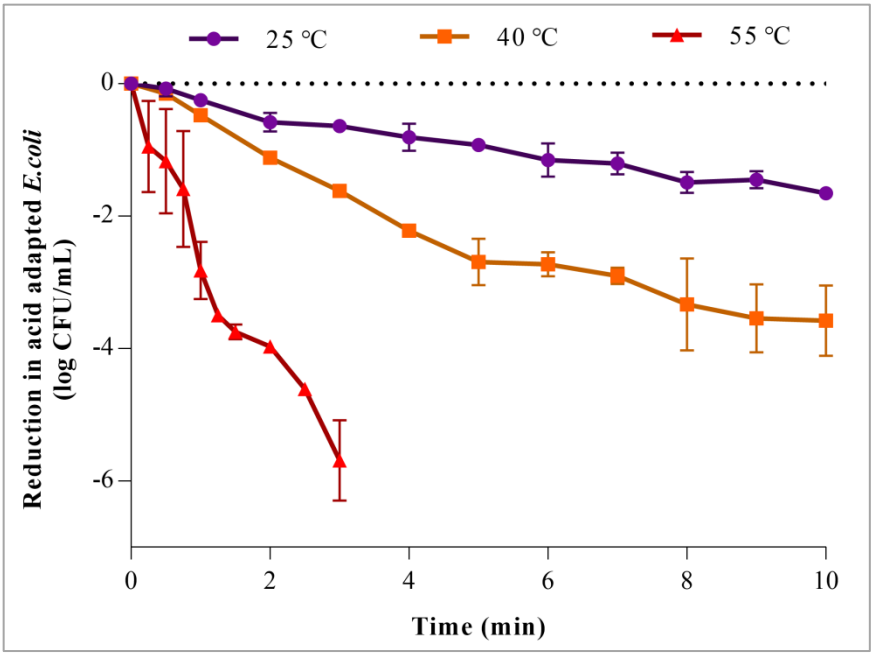


Figure 5.14. Inactivation kinetics of *E.coli* in SJ by US at 25, 40, and 55 °C

The results of this study were in accordance with the other studies. For example, Dincer and Topuz (2015) studied inactivation of *E. coli* ATCC 25922 in black mulberry juice by pulse sonication and continuous thermosonication considering different temperatures. The authors reported the estimated processing time required to achieve 5 log reduction of *E. coli* to be 14.10 min for pulsed sonication at 25 °C and 10.45 min for continuous thermosonication at 50 °C (Dincer & Topuz, 2015). Depending on different processing conditions and *E. coli* surrogate, the sonication time may vary. However, it is remarkable to address the synergistic effect of sonication and mild heating for the achievement of target inactivation levels. Similarly, Salleh-Mack and Roberts (2007) also claimed that sonication increased the sensitivity of *E. coli* (25922) to thermal inactivation (Salleh-Mack & Roberts, 2007). On the other hand, Patil et al. (2009)

related the efficacy of sonication to the level of amplitude, treatment time, and type of media (Patil, Bourke, Kelly, Frias, & Cullen, 2009).

Although application of US was easy to conduct, one of the challenges of US is the temperature control during processing (Rastogi, 2011). Due to the effect of cavitation and energy conversions, the temperature inside the product increases during sonication process. In this study, the temperature of juice was controlled by using a water bath connected to the double wall sample unit. Figure 5.15 shows the temperature change during US processing. Even though the temperature of circulating water was adjusted to 4 °C, the temperature of SJ inside the sample container increased up to 34.55 after 5 min, and levelled out until the end of sonication (10^{th} min). In spite of increase in temperature, the reduction of *E. coli* 11775 cells in SJ was still below 2 log CFU/mL when sub-lethal temperature of 25°C was employed. On the other hand, when the treatment temperatures were kept at 40 °C and 55 °C, the temperature control was much easier by circulating the water at 15 and 25 °C, respectively. The maximum temperature reached by the SJ was 43 °C and 56.48 °C for sonication at 40 °C and 55 °C, respectively.

The acoustic power was calculated as 2003.4 W considering the Equation 5.4 (Baumann, Martin, & Feng, 2005):

$$\text{Power (W)} = m \times C_P \times \frac{dT}{dt} \tag{5.4}$$

where m is the mass of juice (g), Cp is the specific heat (J/g°C) of SJ, and dT/dt is the change in temperature over time. As given in Table 5.5 (in section 5.3.1.1), the specific heat of SJ at 22 °C was measured as 3.71 J/g°C. dT/dt was calculated considering the minimum and maximum temperature of SJ during 3 min of thermosonication. Then the acoustic energy was calculated as 5.15 ± 1.34 J/mL based on Equation 5.5:

$$\text{Acoustic energy (W/mL)} = \frac{P}{V} \tag{5.5}$$

where P is the power obtained by Equation 5.4, and V is the volume of the SJ.

With respect to the decontamination of liquid food products by sonication, the widely used operating conditions in terms of frequency, amplitude % and treatment time

were reported to be 20 kHz, 60-80 %, 5-30 min, respectively. However, considering these conditions, it would not be possible to accomplish FDA's requirement of 5-log reduction of the microorganism of concern (Zinoviadou et al., 2015). In this study, the sonication processing conditions for the pasteurization of SJ at 55 °C in terms of acoustic energy, amplitude (100%), frequency, mode of pulsing and treatment time were determined to be 5.15 ± 1.34 J/mL, 120 µm, 24 kHz, continuous pulsing mode, and 3 min, respectively. Since FDA's 5 log reduction criterion was achieved when the SJ inoculated with acid adapted *E. coli* 11775 was subjected to sonication at mild temperature, the term "thermosonication" was used for the further sections as well as for Chapter 6 and Chapter 7.

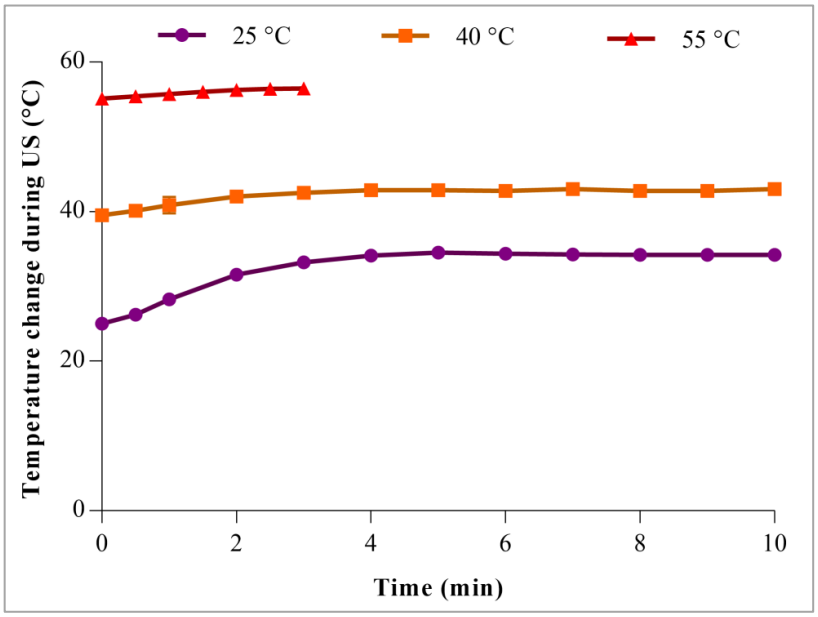


Figure 5.15. Temperature change during US processing of SJ

5.3.5. Inactivation of Acid Adapted *E.coli* in Model Solution by PEF

Variance analysis of response surface quadratic model is given in Table 5.10. The significance of main (x_1 (EFI) and x_2(treatment time)), interaction ($x_1.x_2$) and quadratic ($x_1^2$and x_2^2) effects on inactivation of acid adapted *E. coli* 11775 cells in model solution by PEF were evaluated. Due to small p-values (<0.05), the electrical field intensity (EFI), treatment time (t), interaction of EFI and t, and t^2 were found significant in the model. However, quadratic effect of EFI (x_1^2) was insignificant with a

p-value of 0.1825. Therefore, x_1^2 was removed from the model. In this respect, the reduction of acid adapted *E.coli* cells in MS subjected to PEF was estimated by following equation in coded terms:

$$\text{Reduction } (\log \frac{\text{CFU}}{\text{mL}}) = 2.77 + 1.23 \times x_1 + 1.53 \times x_2 + 0.60 \times x_1 x_2 - 0.43 \times x_2^{\,2} \quad (5.6)$$

The quadratic model was found significant with a non-significant lack-of-fit value. The R^2 and the adjusted R^2 were in a good agreement having the values of 0.9942 and 0.9920, respectively.

Table 5.10. Analysis of variance (ANOVA) for response surface quadratic model

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value**	
Model (R^2:0.9942)	50.18	5	10.04	447.66	< 0.0001	significant
EFI (x_1)	18.24	1	18.24	813.73	< 0.0001	
Treatment time (x_2)	28.19	1	28.19	1257.43	< 0.0001	
$x_1.x_2$	2.85	1	2.85	127.25	< 0.0001	
x_1^2	0.04	1	0.04	1.98	0.1825	
x_2^2	0.81	1	0.81	35.93	< 0.0001	
Residual	0.29	13	0.02			
Lack of Fit	0.11	3	0.04	1.92	0.1896	not significant
Pure Error	0.18	10	0.02			
Cor Total	50.47	18				

Figure 5.16 shows the response surface plot of logarithmic reduction of *E.coli* in MS exposed to PEF processing considering varying EFI (25, 30, and 35 kV/cm) and treatment time (5, 16, and 27 μs). Several processing parameters related to PEF technology have been studied for the determination of inactivation kinetics of microorganisms. However, electrical field intensity and the treatment time have been considered as among the most important parameters that affect inactivation efficacy of microorganisms (Raso, Condón, & Álvarez, 2014). In this study, 35 kV of EFI resulted in complete inactivation of acid adapted *E. coli* 11775 cells (5.53 log CFU/mL) when the MS was subjected to PEF for 27 μs. Here, the treatment time referred to the time that the MS were exposed to the pulsed electric field. On the other hand, the total processing time between the entrance and exit points of the MS in the whole system was recorded as 2.67 min. The results of this study were in line with the findings of

Mosqueda-Melgar et al. (2008). They stated that depending on the characteristics of media, operating parameters, and the target microorganism, the applied EFI would be varied from 20 kV/cm to 80 kV/cm in batch or continuous systems (Mosqueda-Melgar, Elez-Martinez, Raybaudi-Massilia, & Martin-Belloso, 2008). They also reported the treatment time ranges of PEF from 12 to 400 μs for inactivation of *E. coli* in different types of liquid foods (Mosqueda-Melgar et al., 2008). Besides, there are also some other studies that have applied PEF for more than 400 μs. For example, Plaza et al. (2006) subjected orange juice to electrical field at the intensity of 35 kV/cm for 750 μs (Plaza et al., 2006). In another study, orange juice was processed by PEF considering 17 kV/cm of electric field strength for 1034 μs of treatment time (Agcam, Akyildiz, & Evrendilek, 2016). Strawberry juice was processed by PEF considering electrical field intensity of 35 kV/cm for 1700 μs (Aguilo-Aguayo, Oms-Oliu, Soliva-Fortuny, & Martin-Belloso, 2009). The variations in the treatment times among previously published studies could depend on the conductivity of the juice, polarity mode, pulse width etc. (Wouters et al., 2001).

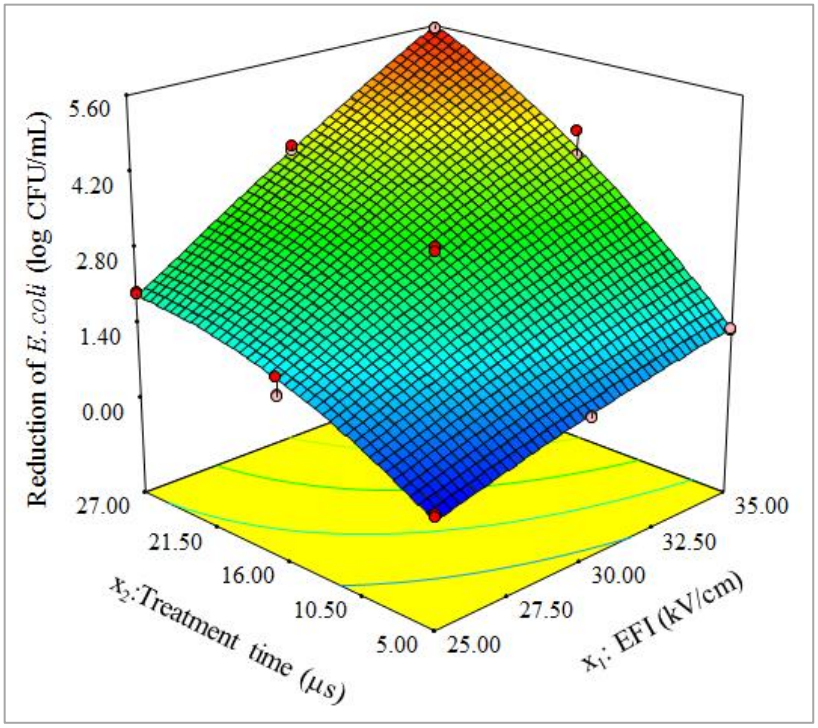


Figure 5.16. Response surface plot of log-reduction of acid adapted *E. coli* 11775 in MS subjected to PEF

As shown in Figure 5.16, the elevated electrical field intensity resulted in an increased effectiveness of PEF treatment on inactivation of acid adapted *E. coli* 11775

cells in SJ. Thus, more reductions of *E. coli* cells were achieved at higher electrical field intensity compared to lower EFI. This is actually attributed to the better disintegration of cell matrices due to the increased intensity of electrical field (Töpfl, 2006).

The number of pulses per transit and frequency were calculated by using Equation 5.7 and Equation 5.8, respectively.

$$\text{Number of pulses per transit} = \frac{t_{dose}}{N \cdot w} \tag{5.7}$$

$$\text{frequency (1/s)} = \frac{t_{dose} \cdot \dot{V}/A}{w \cdot N \cdot L} \tag{5.8}$$

where t_{dose} is the treatment time (μs), N is the number of treatment cells which is 4, and w is the pulse width (μs), $\dot{V}$ is the flow rate (cm^3/s), A is the cell area (cm^2), and L is the length of cell (cm).

Table 5.11. PEF processing parameters for model solution based on central composite design

EFI (kV/cm)	t_{dose} (μs)	Frequency (Hz)	n	T_{in} (°C)	T_{out} (°C)	ΔT (°C)
25	5	29	3	22.7	23.9	1.2
35	5	29	3	22.6	25.9	3.3
25	27	155	14	22.8	31.9	9.1
35	**27**	**155**	**14**	**22.7**	**46.0**	**23.3**
25	16	92	8	22.8	27.6	4.8
35	16	92	8	22.6	34.6	12
30	5	29	3	22.7	24.8	2.1
30	27	155	14	22.6	37.7	15.1
30	16	92	8	22.7	30.8	8.1

Electrical conductivity (3.9 mS/cm), flow rate (350 mL/min), and pulse width (2 μs) were kept constant.
n: total number of pulses in four treatment cells
T_{in}: Temperature of MS at the inlet point
T_{out}: Temperature of MS at the outlet point
$\Delta T = T_{out} - T_{in}$

Table 5.11 shows the processing parameters of PEF technology that was applied to MS based on a central composite design. Corresponding frequencies for the applied t_{dose} of 5, 16, and 27 μs were 29, 92, and 155 Hz, respectively. Accordingly, the total number of pulses applied by 4 treatment cells varied from 3 to 14 pulses depending on the treatment time (t_{dose}). The initial temperature of the MS was approximately 22.7 ±

0.07 °C. Depending on the applied EFI and frequency, thereby t_{dose}, the temperature of model solution at the outlet of the system increased up to 46 °C due to the energy dissipation (Table 5.11). Even though the temperature increased during processing, it was still below the lethal temperature.

The experimental and predicted logarithmic reduction data obtained from the quadratic model using central composite design were compared and shown in Table 5.12. Consequently, 5.53 log CFU/mL reduction of *E.coli* 11775 was obtained when the MS was exposed to PEF at an electrical field of 35 kV/cm, t_{dose} of 27 μs, frequency of 155 Hz, pulse width of 2 μs, flow rate of 350 mL/min, electrical conductivity of 3.9 mS/cm. Thus, FDA's 5-log reduction requirement was satisfied for model solution of strawberry juice under these operating conditions.

Table 5.12. Comparison of experimental and predicted PEF data of *E. coli* based on central composite design

EFI (kV/cm) (x_1)		**t_{dose} (μs) (x_2)**		**Reduction in *E.coli* cells (log CFU/mL)**	
Actual	**Coded**	**Actual**	**Coded**	**Experimental**	**Predicted**
25	-1	5	-1	0.18	0.17
25	-1	5	-1	0.14	0.17
35	+1	5	-1	1.29	1.44
35	+1	5	-1	1.33	1.44
25	-1	27	+1	2.02	2.04
25	-1	27	+1	1.97	2.04
35	**+1**	**27**	**+1**	**5.53**	**5.70**
35	**+1**	**27**	**+1**	**5.53**	**5.70**
25	-1	16	0	1.12	1.53
25	-1	16	0	1.45	1.53
35	+1	16	0	3.75	4.00
35	+1	16	0	4.23	4.00
30	0	5	-1	0.76	0.80
30	0	5	-1	0.72	0.80
30	0	27	+1	3.83	3.87
30	0	27	+1	3.93	3.87
30	0	16	0	2.87	2.77
30	0	16	0	2.77	2.77
30	0	16	0	2.77	2.77

When the experimental data was plotted versus predicted data as shown in Figure 5.17 a good linear correlation was obtained (R^2 value is 0.9933). This means that

the model well described the experimental data. In literature, response surface methodology has been successfully applied for the evaluation of inactivation of either pathogenic or spoilage microorganisms in different types of media (Gomez, Garcia, Alvarez, Condon, & Raso, 2005; Marselles-Fontanet, Puig, Olmos, Minguez-Sanz, & Martin-Belloso, 2009). It would worth to mention that PEF processing should be carefully carried out in order to avoid arching, pumping any possible air bubbles to the treatment chamber, or exceeding temperatures above limitation. Otherwise, the PEF system might stop and quit pulsing while the liquid passes through the chamber. In that case, some liquids would be passed through the system without receiving the applied electrical field. Accordingly, the EFI, the residence time, and the distribution of microorganisms throughout the product must be homogeneous and uniform inside the treatment chamber in order to let the microbial cells to receive same intensity.

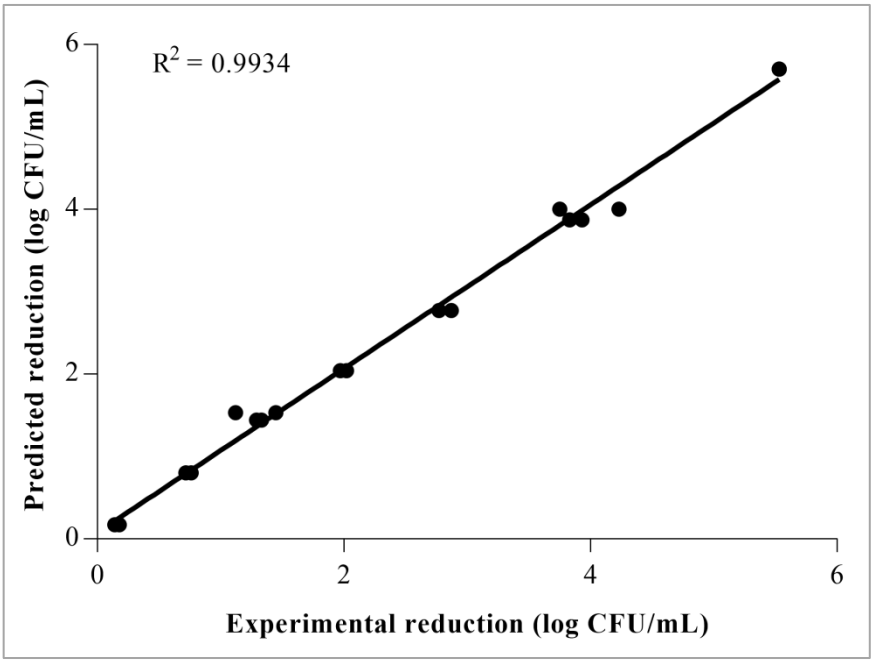


Figure 5.17. Experimental versus predicted data for the reduction of *E. coli* in SJ

5.3.6. Inactivation of Acid Adapted *E. coli* by Thermal Processing

Acid-adapted *E. coli* O157:H7 has been reported to have greater heat resistance than *Salmonella* and *L. monocytogenes* as studied in apple, orange, and white grape juice (Mazzotta, 2001). As a surrogate of *E. coli* O157:H7, a complete inactivation of *E. coli* 11775 cells was achieved in SJ by thermal pasteurization at 71.7 °C for 15 s. Thus,

FDA's criterion of 5-log reduction in the pertinent microorganism was successfully satisfied in the present study. Hence, the thermal pasteurization conditions for SJ were selected as 71.7 °C for 15 s on the basis of reduction of *E. coli* 11775 equivalent to that of other studied nonthermal technologies. Kaya et al. (2015) used *E. coli* K-12 as a surrogate for the determination of thermal pasteurization conditions for lemon melon juice blend. The authors achieved approximately 6 log reduction of *E. coli* K-12 in the juice blend subjected to thermal pasteurization at 72 °C for 71 s (Kaya, Yildiz, & Unluturk, 2015).

5.3.7. Validation of Processing Conditions for SJ

Freshly squeezed SJ was exposed to HPP, US, PEF and thermal processing under the equivalent conditions that were obtained in the previous studies. For this purpose, the freshly squeezed strawberry juice was kept at 4 °C for 5 days in order to increase its natural microbial flora

High Pressure Processing

Figure 5.18 shows the logarithmic reduction of total mesophilic aerobic count (TMAC) and yeast mold (YM) in SJ treated with HPP. The initial population of TMAC was 3.68 ± 0.30 log CFU/mL. After processing the juice at 300 MPa for 1 min, 1.81 ± 0.15 log CFU/mL reduction was obtained. On the other hand, HPP at 350 MPa for 30 s and 400 MPa for 15 s resulted in 1.60 ± 0.03 and 1.56 ± 0.16 log CFU/mL reduction of TMAC, respectively. However, statistically, no significant difference was found among HPP treatments (300 MPa – 1 min; 350 MPa – 30 s; 400 MPa – 15 s) in terms of TMAC of SJ. Similar trend was also observed for yeast-mold (YM) count recorded before and after HPP under the same conditions. The initial YM count of strawberry juice was 5.25 ± 0.20 log CFU/mL. The highest reduction of YM (4.07 ± 0.58 log CFU/mL) was obtained when the juice processed at 300 MPa for 1 min. The reduction of YM count in SJ processed at 350 MPa for 30 s and 400 MPa for 15 s was 3.42 ± 0.16 and 3.44 ± 0.09 log CFU/mL, respectively. However, there was no significant difference among treatments ($p<0.05$). It was observed that mesophilic aerobic bacteria in SJ were more resistant to HPP processing than yeast and molds in the current study. This finding had correlation with the pressure sensitivities of microorganisms reported

by Fonberg-Broczek et al. (2005). The authors claimed that the prokaryotes were more resistant to pressure compared to yeast and molds (Fonberg-Broczek et al., 2005). On the other hand, it was reported that HPP could increase the sensitivity of *E. coli* O157:H7 to the acidic conditions by resulting in less survival cells after processing (Bull et al., 2004).

High pressure processing times that were commonly used for treatment of liquid foods were reported to be up to 5 min (Guerrero-Beltran et al., 2011; Ramaswamy et al., 2003). This was in line with this study. Similarly, Jayathunge et al. (2015) pasteurized tomato juice by HPP at 600 MPa for 1 min at ambient temperature (approximately 16 °C) (Jayathunge, Grant, Linton, Patterson, & Koidis, 2015). Yi et al (2017) subjected cloudy apple juice to 600 MPa for 3 min (Yi et al., 2017). Although the use of pump for such low times for compressing, holding and decompressing may reduce the life of pump, processing times less than 1 min may not be preferred. In order to guarantee the microbial safety and provide applicability, 300 MPa and 1 min at around 20 °C were selected as the final HPP conditions for pasteurization of SJ.

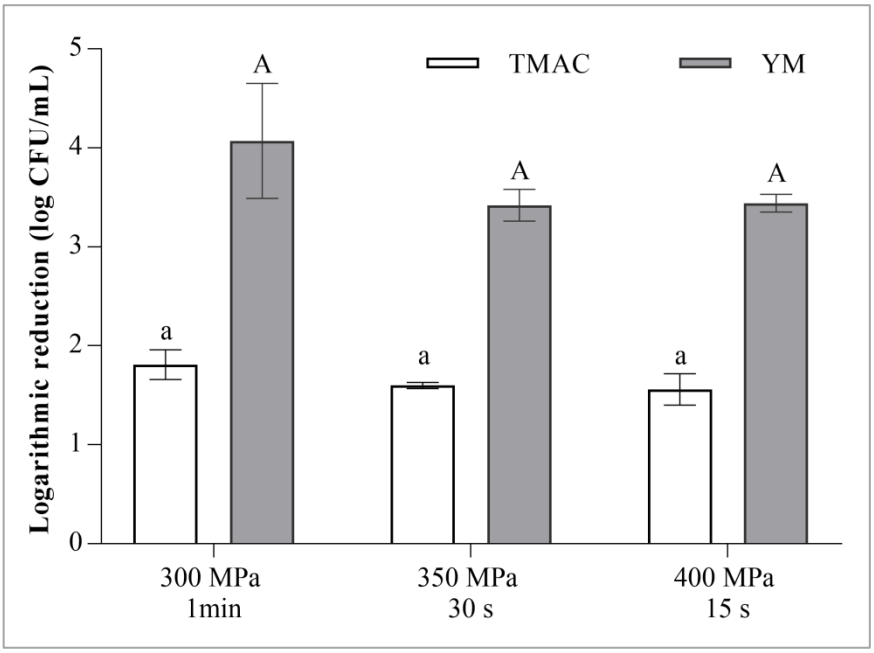


Figure 5.18. Logarithmic reductions in total mesophilic aerobic count (TMAC) and yeast-mold count (YM) in SJ treated by HPP
(Different letters shows statistically significant differences. No significant difference was observed for TMAC (small case letters) and YM (capital case letters))

Thermosonication

Thermosonication conditions which resulted in 5.69 ± 0.61 log CFU/mL reduction of acid adapted *E.coli* cells in SJ were applied to inactivate the natural microbial flora of SJ. The sonication at 120 μm of amplitude in continuous pulsing mode and 55 °C for 3 min was able to reduce total mesophilic aerobic count (TMAC) and yeast-mold (YM) count in SJ by 2.72 ± 0.06 log CFU/mL; and 4.06 ± 0.22 log CFU/mL, respectively. Adekunte et al. (2010) studied a specific yeast strain, i.e. *Pichia fermentans*, in order to evaluate the efficacy of sonication for tomato juice. The authors applied varying amplitude levels from 24.4 to 61.0 μm and treatment times from 2 to 10 min considering pulse duration of 5 s on and 5 s off. Similarly, they observed significant reduction in yeast strain as the amplitude and processing time increased (Adekunte, Tiwari, Scannell, Cullen, & O'Donnell, 2010). Gabriel (2012), on the other hand, studied both pathogen bacteria and spoilage yeasts in cloudy apple juice subjected to sonication. They used a mixture of spoilage yeasts containing *Debaryomyces hansenii, Torulaspora delbrueckii, Clavispora lusitaniae, Pichia fermentans* and *Saccharomyces cerevisiae.* The authors stated that the yeasts showed more resistance to sonication compared to acid adapted or non-adapted pathogen bacteria such as *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* (Gabriel, 2012a). These findings are in accordance with the results obtained for strawberry juice in this study. In conclusion, the natural microbial flora of SJ showed more resistance to thermosonication compared to the acid adapted *E.coli* 11775. A similar trend was also observed by Bermúdez-Aguirre et al. (2009). The authors found out that thermosonication of milk at 63 °C for 10 min considering 100 % amplitude resulted in more than 5 log reductions in L. innocua while the same conditions achieved 3.13 log CFU/mL of total mesophilic bacteria (Bermúdez-Aguirre, Corradini, Mawson, & Barbosa-Cánovas, 2009)

Pulsed Electric Field

PEF processing conditions which resulted in 5-log reduction of *E. coli* 11775 in model buffer solution were firstly confirmed by applying the same conditions to the strawberry juice. Figure 5.19 shows the logarithmic reduction of acid adapted *E.coli* in SJ treated by PEF in the same way as it was done for model solution. The effect of PEF on the inactivation of *E. coli* in model solution and SJ were similar. Acid adapted *E.coli* in MS and SJ subjected to PEF were reduced by 5.53 ± 0.00 and 5.16 ± 0.15 log

CFU/mL, respectively. Statistically, there were no significant differences between the logarithmic reduction level of *E. coli* in MS and SJ. Thus, the final PEF processing conditions including electrical field intensity, treatment time, frequency, flow rate, pulse width and mode were determined to be 35 kV/cm,27 μs, 155 Hz, 350 mL/min, 2 μs and monopolar mode, respectively These results were in agreement with the study of Evrendilek et al. (2000). Evrendilek et al. (2000) applied electric field strength of 34 kV/cm for 160 μs considering pulse duration time of 4 μs, frequency of 800 pps in a pilot plant scale PEF system, and achieved 4.5 log reduction of *E. coli* O157:H7 (35150) in apple cider (Evrendilek et al., 2000). Vega-Mercado et al. (1996) reported 2.2 log reductions of *E. coli* 11229 in simulated milk processed at electrical field intensity ranged from 20 to 55 kV/cm. The authors stated that the electrical field strength related to the poration rate and physical damage in the cell membrane (Vega-Mercado et al., 1996). Formation of irreversible pores would result in disintegration of membrane and loss of cell viability when the applied electrical field was higher than the critical value (Saulis, 2010). It was reported that Gram-positive and Gram-negative bacteria were more resistant than the yeasts in general. Due to the morphological changes, larger cells could be more easily permeabilized compared to smaller cells (Wouters et al., 2001). However, a contradictive finding was obtained in the current study. In addition to *E. coli* cells, the efficacy of equivalent PEF processing conditions was tested on natural microbial flora, i.e., TMAC and yeast-mold count of SJ. Although the number of *E.coli* cells was reduced by 5.13 ± 0.15 log CFU/mL at 35 kV/cm and 155 Hz, the reduction in TMAC and YM counts were 2.90 ± 0.25 and 3.69 ± 0.07 log CFU/mL, respectively. Although the total mesophilic aerobic bacteria exhibited more resistance to PEF processing compared to yeast and molds, the inactivation levels of TMAC and YM count were below than that of *E. coli* cells. This result may be attributed to the type of species of bacteria in the mixed population where the microbial load was intentionally increased by keeping the juice at refrigerated conditions for 5 days before processing. Besides, change in the viscosity and formation of any metabolic products during incubation, i.e. fermentation, of SJ can change the homogeneity of the electrical field. Nevertheless, FDA's requirement of 5-log reduction in the population of target microorganism was satisfied and a considerable amount of reduction was achieved for TMAC and YM count. Thus, these conditions were confirmed and chosen as PEF processing condition for pasteurization of strawberry juice.

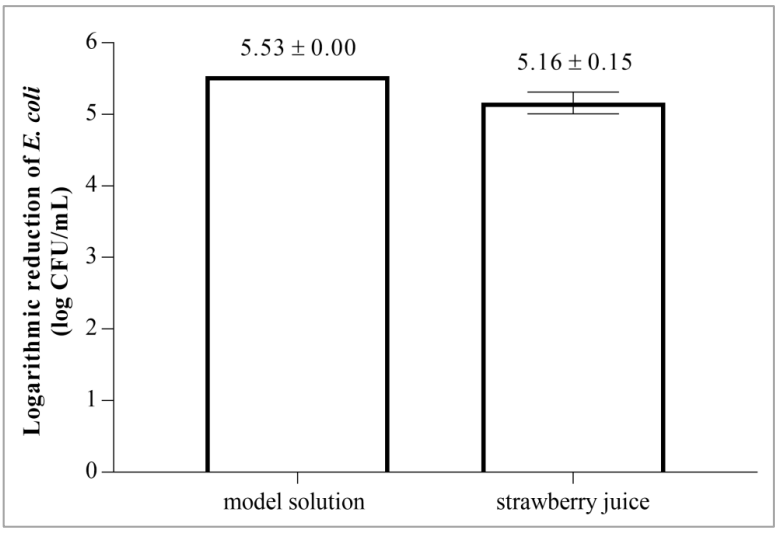


Figure 5.19. The effect of PEF processing on the inactivation of acid adapted *E.coli* 11775 in MS and SJ

Thermal Pasteurization

The temperature-time parameters that resulted in complete inactivation of acid adapted *E. coli* 11775 in SJ were confirmed by treating freshly squeezed SJ having increased level of natural microbial flora. The initial load of total mesophilic aerobic bacteria and yeast-mold in SJ were 4.82 ± 0.61and 5.86 ± 0.50 log CFU/mL, respectively. Thermal pasteurization at 71.7 °C for 15 s resulted in 4.1 ± 0.10 and 5.11 ± 0.15 log CFU/mL reductions of TMAC and YM count, respectively. Since Turkish Food Codex declared the maximum acceptable load of TMAC and YM as 4 log and 3 log CFU/mL (Codex, 2001), thermally pasteurized strawberry juice can be considered as a safe product in terms of natural flora. Similarly, Ahzuvalapril et al. (2010) applied thermal processing to the apple cider inoculated with *E. coli* K-12 at an initial load of approximately 7-log CFU/mL. The authors found that thermal treatments at 72, 74, and 76 °C for 1.3 s at a flow rate of 15 L/h resulted in significant reduction of *E. coli* K-12 population in apple cider (Ahzuvalapril et al., 2010). In conclusion, thermal pasteurization conditions (71.7 °C for 15 s) yielded at least 5 log reduction of *E. coli* in SJ were also resulted in significant reduction in the natural microbial flora of SJ.

As a summary of this section, the effectiveness of thermal and non-thermal technologies applied for the pasteurization of SJ was illustrated in Figure 5.20. The inactivation levels of each treatment were summarized in this figure in terms of TMAC and YM count.

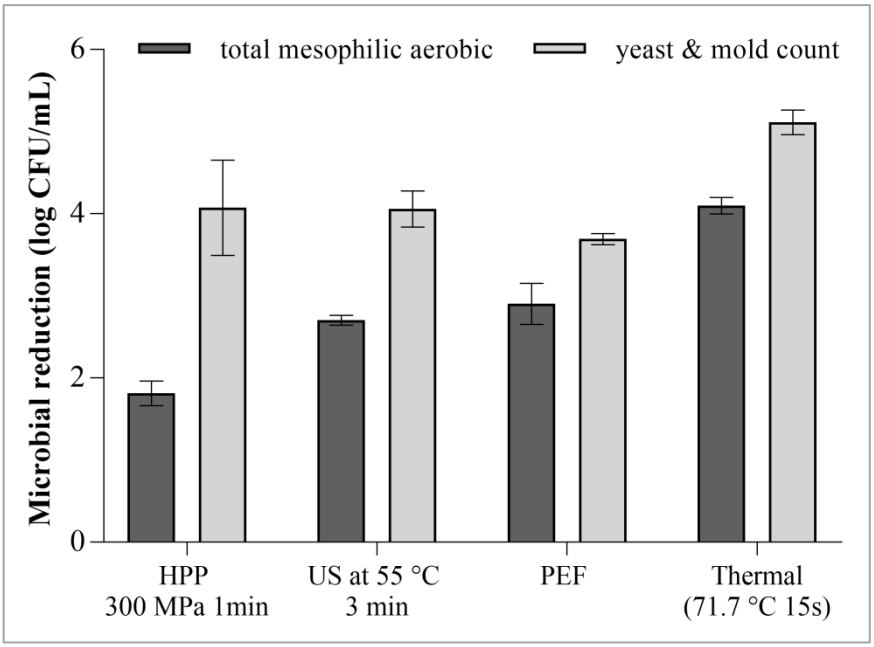


Figure 5.20. Comparison of thermal and nonthermal treatments for mild pasteurization of strawberry juice

5.4. Conclusions

In literature, many researchers investigated the effect of nonthermal processing technologies on juice quality. In order to fairly compare the properties of final products, the juice should be processed under equivalent conditions. Thus, in this part of PhD thesis, the processing conditions for individual or combined technologies were determined in consideration of equivalent processing approach. Firstly, a target microorganism, i.e. acid adapted *E. coli* 11775, was selected based on the survival behaviors of different surrogates in strawberry juice. Then the juice inoculated with acid adapted *E. coli* 11775 was subjected to several processes, i.e. HPP, sonication, PEF, and thermal pasteurization at varying conditions specific to each technology. The processing conditions provided equivalent microbial reductions of at least 5 log cycles in SJ were determined as the final pasteurization conditions for SJ.

In this respect, HPP at **300 MPa for 1 min** were sufficient for achieving 5 log reductions in the number of target microorganism. Ultrasonication, on the other hand, was ineffective to achieve 5-log reduction of target cells at 25 °C. Therefore, hurdle concept was applied by combining mild heat treatment at 55 °C. Thus, thermosonication at **55 °C for 3 min** provided 5.69 ± 0.61 log CFU/mL reduction in the number of *E. coli* population under the conditions of **5.15 ± 1.34 J/mL acoustic energy, 100 %**

amplitude (120 μm), continuous pulsing mode. For the PEF treatment, SJ was successfully simulated by the model solution prepared with citric acid - Na_2HPO_4 buffer solution, glucose, fructose, and salt. Electrical conductivity, pH, and total soluble content (° Brix) of model buffer solution were well adjusted to that of SJ. Both liquids showed similar properties in terms of thermal and rheological properties as well. Newtonian flow behavior was observed for SJ and model solution. There was no significant change between two samples in terms of their specific heat capacity (Cp). A central composite design was applied to study the effect of PEF on the inactivation of acid adapted *E.coli* in MS. Application of PEF under the conditions of **35 kV/cm electrical field intensity, 27 μs treatment time, 155 Hz frequency, 350 mL/min flow rate, 2 μs pulse width in monopolar mode** resulted in 5.53±0.00 log CFU/mL reduction of *E.coli* in MS. This finding was confirmed by exposing SJ inoculated with acid adapted *E.coli* to the same PEF processing conditions. 5.13±0.15 log CFU/mL reduction of acid adapted *E.coli* was obtained in SJ. Thereby, the PEF processing conditions were satisfied for both SJ and MS. Using a model system was a satisfactory approach to select proper conditions for PEF processing of SJ by centering the analysis in the most influential components, and thus saving time and unnecessary guessing. Thermal pasteurization at **71.7 °C for 15 s**, on the other hand, accomplished complete inactivation of acid adapted *E.coli* cells in SJ. Finally, all the equivalent processing conditions accomplished at least 5-log reduction of acid adapted *E.coli* cells in SJ were tested on freshly squeezed and fermented SJ to reveal their effect on the inactivation of natural microbial flora of SJ. Since remarkable reductions were obtained in TMAC and YM counts of SJ after subjecting the juice to equivalent pasteurization, the processing conditions were confirmed and declared as the final pasteurization conditions.

This chapter demonstrated that the equivalent processing approach was well applied for the determination of mild pasteurization conditions for strawberry juice. Accordingly, thermal and nonthermal processing conditions which revealed at least 5-log reduction of acid adapted *E. coli* 11775 was considered as the final processing parameters for pasteurization of strawberry juice at moderate temperatures. Since the microbial safety of food product is the major concern in food processing, the processing conditions for each technology must satisfy FDA's regulation of 5-log reduction in the microorganism of concern in fruit and vegetable juices. Food quality affected by different processes should be investigated once the similar safety conditions are

fulfilled. In this regard, next chapter will focus of the evaluation of the physicochemical and phytochemical properties of SJ pasteurized by HPP, thermosonication, PEF, and thermal treatment under equivalent conditions.

CHAPTER 6

OVERALL QUALITY ATTRIBUTES AND RETENTION OF BIOACTIVE COMPOUNDS OF STRAWBERRY JUICE PASTEURIZED UNDER EQUIVALENT PROCESSING CONDITIONS

6.1. Introduction

Nonthermal food processing technologies have been gained special attention in order to avoid negative impact of thermal pasteurization on quality of fruit juices (Sanchez-Moreno, De Ancos, Plaza, Elez-Martinez, & Cano, 2009). In literature, many studies have been conducted on mild preservation of fruit juices by innovative nonthermal processing technologies. However, the variances arise from the use of raw material with varying initial quality, as well as different maturity levels, processing and storage conditions make the case difficult to compare with other processes. Therefore, the equivalent processing approach was proposed considering equivalent products in terms of inactivated microorganisms or enzymes (Timmermans et al., 2011). Equivalent degree of microbial inactivation constitutes a fair basis for the comparison of chemical and biochemical quality parameters of the final product (Vervoort et al., 2011).

In this chapter, the microbial quality as well as physicochemical and phytochemical properties of freshly squeezed strawberry juice (SJ) subjected to thermal and nonthermal processing technologies were evaluated. The juice was pasteurized by high pressure processing (HPP), thermosonication (US), pulsed electric fields (PEF), and conventional thermal pasteurization under equivalent processing conditions determined in Chapter 5 considering same level of reduction of acid adapted *E.coli* 11755 (≥ 5 log CFU/mL). The strawberry juice subjected to equivalent processing conditions were then used for further experiments to assess the microbial quality, pH, titratable acidity, total soluble solids (TSS) (°Brix) of SJ before and after the treatments. Moreover, the phytochemical properties such as total phenolic content (TPC), total anthocyanin content (TAC), and antioxidant activity of SJ samples were evaluated as an

indicator of retention of bioactive compounds in the juice. Equivalent processing approach enabled to make a fair comparison of different quality parameters of SJ pasteurized with different technologies.

6.2. Material and Methods

6.2.1. Equivalent Pasteurization of Strawberry Juice

Freshly squeezed strawberry juice was prepared as previously described in Chapter 4, and then pasteurized by thermal and nonthermal technologies. In this chapter, freshly squeezed SJ was directly subjected to HPP, thermosonication, PEF or thermal treatment under the equivalent conditions that were previously determined in Chapter 5. Here, a summary of the treatments were given.

A high pressure unit from Engineering Pressure Systems, Inc., (Andover, MA, USA) was used for HPP treatment. Freshly squeezed SJ was packed in the plastic pouches (200 mL) (Nasco Whirl-Pak™, Fort Atkinson, WI). The pouches were then carefully sealed and placed into the cylindrical chamber vessel containing the pressure transmitting liquid. HPP was then applied to SJ at 300 MPa for 1 min in duplicate. The temperature and pressure profile of the process were monitored during treatment. It took 0.5 s to come up to the desired pressure, and less than 0.5 s to depressurize. The initial temperature of the pressurizing liquid inside the chamber (18.33 ± 1.04 °C) reached to maximum temperature of 26.13 ± 1.12 °C during holding period. Thus, the temperature rise during compression was 2.6 °C/100 MPa.

US was combined with mild heating in order to increase the lethal effect of sonication. Therefore, this treatment was renamed as thermosonication throughout this chapter. An ultrasonic device Hielscher (Hielscher USA Inc., Ringwood, NJ) equipped with a probe of 22 mm diameter, double wall sample unit and a water bath (Viscotherm VT 10) was used for the pasteurization of SJ. Four hundred mL of freshly squeezed SJ, which had initial temperature of 25 °C, was placed into a double-wall glass sample unit. Two K-type probes were immersed into the sample container at different locations in order to monitor the temperature increase during sonication. The temperature of water circulating around the sample unit was adjusted to 25 °C in order to keep the thermosonication temperature at around 55 °C. Once the sonicator was turned on, the

temperature of SJ increased due the energy transmission into the sample. After a certain come-up-time (4.40 s), the temperature of SJ was reached to 55 °C. At that point, the treatment time for thermosonication started and recorded manually by a chronometer. The duplicate juice samples were subjected to thermosonication considering 5.15 ± 1.34 J/mL of acoustic energy, 100 % amplitude (120 μm) in continuous pulsing mode at 55 °C for 3 min of treatment time.

PEF, on the other hand, was conducted by using a pilot plant scale Powermod^{TM} PEF system (Diversified Technologies Inc., Bedford, MA, USA) equipped with two pairs of co-field treatment chambers. Six liters of freshly squeezed SJ placed in jar was connected to PEF processing line. The juice with an initial temperature of 22.7 °C was passed through the PEF treatment chamber by a peristaltic pump. The freshly squeezed SJ was subjected to PEF treatment that was applied using monopolar pulses of 2 μs duration at electric field intensity (EFI) of 35 kV/cm, treatment time of 27 μs, frequency of 155 Hz, flow rate of 350 mL/min. EFI, frequency, and pulse width was adjusted by using the control panel of PEF system. The temperature of juice was measured at the inlet and outlet of treatment chamber using K-type thermocouple (Omega Engineering Inc., Stamford, CT). The temperature of SJ at the inlet and outlet points of PEF treatment chamber was recorded as 22.7 and 46 °C, respectively.

A conventional thermal pasteurization was carried out in a double walled sample unit connected to a water bath (Viscotherm VT 10). Four hundred mL of SJ was poured into the sample container. Then, the sample unit containing the juice and a magnetic stir bar was placed on a magnetic stirrer. The speed of stirrer was adjusted to 250 rpm in order to increase the heat transfer inside the medium. The juice was heated up to 71.7 °C by circulating hot water at 74 °C between double walls of sample unit. Once the juice reached to the desired temperature, mild heating of SJ was carried out at 71.7 °C for 15 s. The temperature was controlled by K-type thermocouple. The treated juice was then placed into a previously sterilized bottle and immediately cooled down by placing into iced water.

All of the samples treated by each technology were kept at 4 °C prior to analysis, i.e. microbial, physicochemical and phytochemical attributes of SJ. The details of such assays were given in Chapter 4.

6.2.2. Overall Quality Attributes and Phytochemical Properties of Strawberry Juice

Microbial quality of strawberry juice samples before and after processing was evaluated in terms of total mesophilic aerobic count (TMAC) and yeast and mold (YM) count. The samples were plated on Plate Count Agar (PCA), Potato Dextrose Agar (PDA) acidified with 10 % of tartaric acid by pour plate method for TMAC and YM count, respectively. The plates were then incubated at 37 °C for 48 h for TMAC, and 25 °C for 5 days for YM count. After appropriate time of incubation, the number of viable cells was counted, and the results were expressed as log CFU/mL. Untreated SJ was used as control.

Regarding the physicochemical properties, pH, total soluble solids, and titratable acidity of SJ were measured. Briefly, pH measurement was carried out by using 10 mL of SJ and a bench top pH meter (Mettler Toledo™ FE20 FiveEasy) at 22°C. A digital hand-held refractometer (PAL-α, Atago CO., LTD.) was used to determine the total soluble solids (°Brix) of the samples (3-4 drops). Titratable acidity, on the other hand, was determined according to Cemeroglu (2010) by titrating 10 mL of SJ against 0.1 N NaOH up to pH 8.1. The acidity (%) was then calculated considering the normality of NaOH, normality factor, miliequivalent weight of citric acid, the volume of NaOH used for titration.

Phytochemical properties of bioactive compounds including total phenolic content (TPC), total anthocyanin content (TAC), and antioxidant activity of SJ were also determined. Total phenolic contents of SJ samples were determined according to the Folin-Ciocalteu method (Singleton & Rossi, 1965; Tezcan, Gultekin-Ozguven, Diken, Ozcelik, & Erim, 2009) by using HP 8452A diode array spectrophotometer (Agilent Technologies, Palo Alto, USA). The concentrations of total phenolic compounds in SJ samples were expressed as mg gallic acid equivalents (GAE) per L. A pH differential method was used for determination of the anthocyanin content of strawberry juice (Meyers, Watkins, Pritts, & Liu, 2003). SJ samples prepared in two different buffers at pH values of 1.0 and 4.5 were spectrophotometrically measured at 510 and 700 nm. Then, the results were expressed as mg pelargonidin-3-glucoside per L. Free-radical scavenging capacity of SJ samples was determined on the basis of DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Odriozola-Serrano, Soliva-Fortuny, &

Martin-Belloso, 2009). Antioxidant activities of the samples were given as % inhibition as detailed in Chapter 4.

6.2.3. Data Analysis

All the tests related to microbial quality and physicochemical properties were performed in duplicate. Phytochemical properties, on the other hand, conducted in triplicate.

Changes in the quality parameters (microbial, physicochemical and phytochemical properties) for the different treatments (untreated, thermal, HPP, US, PEF) were quantified. Differences between the various treatments compared in this study were expressed as mean ± standard deviation (SD). Data analysis was conducted by using Excel worksheet (Microsoft Office 2010, USA) and Minitab 16 software (Minitab Inc., State College, PA, USA). Variance analysis (ANOVA) followed by Tukey comparison test was performed considering 95 % of confidence interval ($p<0.05$). Multivariate data analysis including principal component analysis (PCA) and hierarchical cluster analysis (HCA) were utilized to classify the SJ samples subjected to different treatments in terms of their physicochemical and phytochemical properties. PCA and HCA were conducted by Minitab 16.0 software to visualize the data structure.

In this context, a data matrix was constructed using the analyzed physicochemical and phytochemical properties of SJ samples as columns and pasteurization technologies as rows. The data matrix was introduced into Minitab 16 data analysis software (Minitab Inc., State College, PA, USA). Computation of PCA was conducted considering correlation type of matrix and 5 components. The score values, coefficients, as well as eigenvalues were saved as storage data. Score and loading plots were drawn as PCA output. PCA enabled the reorganization of the information in the data set of the samples. Finally, freshly squeezed SJ samples subjected to different pasteurization technologies were classified in terms of their physicochemical and phytochemical properties.

The cluster analysis was applied to classify untreated (control) and pasteurized SJ samples in terms of their physicochemical and phytochemical properties. Accordingly, the PC-scores of interest were loaded to Minitab 16 data analysis software as new input data for HCA. The computation was carried out considering Ward's

linkage as amalgamation method and Euclidean distance as similarity measurement. Finally, the similarities/dissimilarities among the pasteurization technologies and control SJ were plotted as dendrogram. The interpretation of the samples on a tree-shaped map was conducted considering pH, titratable acidity, TSS, total phenolic content, antioxidant capacity, total anthocyanin content of SJ samples.

6.3. Results and Discussion

6.3.1. Microbial Quality of SJ

Microbiological analysis showed that untreated juice samples had an initial load of 3.11 ± 0.09 log CFU/mL total mesophilic aerobic plate count (TMAC) and 3.41 ± 0.04 log CFU/mL yeast-mold (YM) count. TMAC and yeast-mold count of strawberry juice was reduced below 2 log CFU/mL after processing of SJ by thermal pasteurization, thermosonication, HPP and PEF (Figure 6.1). Among the all processes, thermal pasteurization resulted in the highest reduction in both total mesophilic aerobic and yeast and mold counts. However, statistically there was no significant difference among pasteurization technologies in terms of TMAC and YM counts of treated SJ samples.

Bull et al. (2004) processed Valencia and Navel orange juices by HPP. The initial viable counts for total aerobic and yeast-mold counts in Valencia orange juice and Navel orange juice were 7.8 and 4.8 log CFU/mL; and 4.5 and 3.1 log CFU/mL, respectively. The authors reported that HPP at 600 MPa, 20 °C for 60 s reduced the microbial load to non-detectable levels in Valencia and Navel orange juices (Bull et al., 2004). Cao et al. (2014) studied inactivation of total aerobic bacteria and yeast-mold by HPP in both cloudy and clear apple juices at 600 MPa and varying processing times up to 6 min. Regardless of time, the authors achieved complete inactivation of total aerobic bacteria and yeast-molds in both type of juices when the initial microbial load was less than 4 log CFU/mL. It was stated that cloudy strawberry juice required more time (4 min) in order to reduce the total aerobic bacteria count below the reported detection limit (10 CFU/mL) (Cao, Liu, Wu, Liao, & Hu, 2014). In another study, high pressure processing of carrot juice at 500 and 600 MPa for 1 min at 20 °C reduced total plate count by approximately 4 log CFU/mL (Patterson, Mckay, Connolly, & Linton, 2012).

Even though microbial reductions obtained for SJ in the current thesis were found less than previously mentioned studies, it is still remarkable to provide microbiologically safe product after HPP at 300 MPa for 1 min.

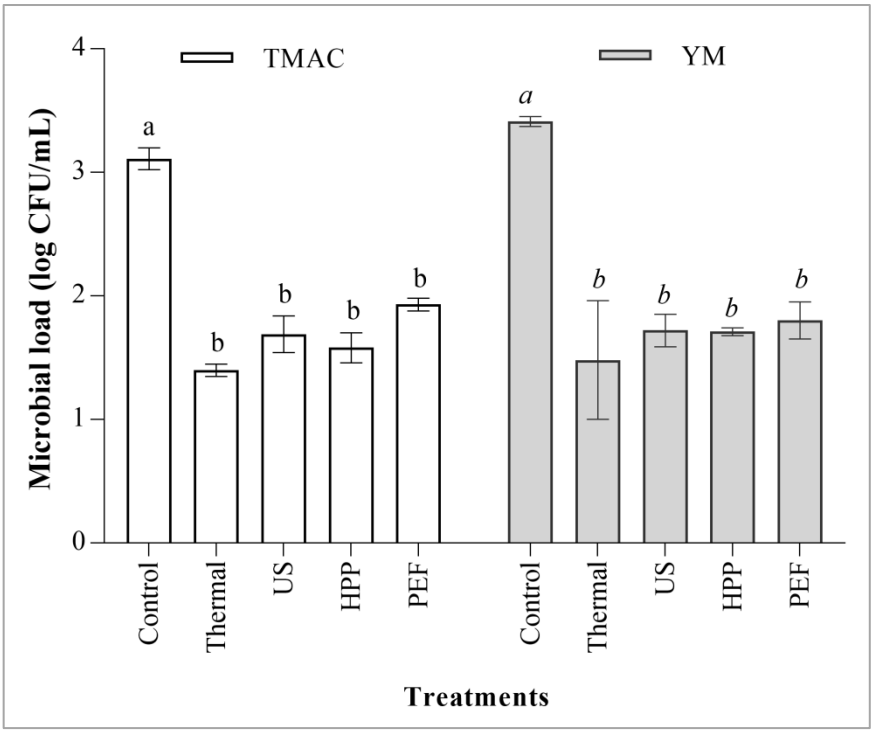


Figure 6.1.Total mesophilic aerobic (TMAC) and yeast-mold (YM) counts in SJ samples before and after processing
(Different letters show significant differences among treatments in terms of TMAC and YM count in SJ samples. Small case letters belong to TMAC while *italic* small case letters refer to YM count.)

Elez-Martinez (2006) applied high intensity PEF (35 kV/cm for 1,000 μs with 4-μs bipolar pulses at 200 Hz) and thermal treatment (90 °C for 1 min) to orange juice having an initial microbial load of 3.5 and 2.9 log CFU/mL of total aerobics and yeast and mold counts, respectively. The authors reduced the initial microbial counts below 1 log CFU/mL after processing with either thermal or PEF processing (Elez-Martinez, Soliva-Fortuny, & Martin-Belloso, 2006). Walkling et al. (2010) applied PEF (34 kV/cm, 60 μs) in combination with mild heat (55 °C, 60 s) for a fruit smoothie. Natural flora of the smoothie was reported to be reduced to 1.9 log CFU/mL and 1.1 log CFU/mL in terms of total aerobic and yeast-mold counts, respectively (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2010). In accordance with these studies, TMAC and YM count was reduced to below 2 log CFU/mL after PEF treatment in this thesis. Combination of high intensity PEF with some antimicrobial agents such as citric

acid, cinnamon bark oil was also suggested as an alternative preservation method for strawberry, orange, apple, pear and tomato juices (Mosqueda-Melgar, Raybaudi-Massilia, & Martin-Belloso, 2012).

Ultrasound processing can reduce the microbial load due to acoustic cavitation that can damage biological species by increasing local temperature and pressure inside the product (Abid et al., 2013). Bhat and Goh (2017) treated strawberry juice by sonication considering 25 kHz frequency with a power set at 70 % (at 20 °C) at varying sonication times up to 30 min. The initial counts for total aerobic bacteria and yeast and molds were 4.70 and 4.66 log CFU/mL, respectively. However, the authors were not able to achieve a significant degree of inactivation for the total mesophilic aerobic bacteria and yeast and molds after sonication (Bhat & Goh, 2017). This was attributed to the resistance of microorganisms, presence of spores of bacteria or fungi (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). It was reported that, in general, fungi might offer higher resistant compared to bacteria due to the cell wall composition (Chemat, Zill-e-Huma, & Khan, 2011). Thus, a combination sonication with mild heating can be considered in order to increase the lethal effect of the treatment. In the case of this study, thermosonication was successfully applied for mild pasteurization of strawberry juice. Accordingly, TMAC and YM count was reduced to below 2 log CFU/mL by thermosonication.

Thermal pasteurization at 71.7 °C for 15 s reduced the TMAC and YM count below 2 log CFU/mL. This finding was in the line with previously published studies. Bull et al (2004) reduced the total aerobic bacteria from 7.8 to 4.3 log CFU/mL and YM from 4.8 to 3.0 log CFU/mL in orange juice after applying a thermal treatment at 65 °C for 60 s (Bull et al., 2004). Elez-Martinez et al. (2006) applied conventional thermal pasteurization at 90 °C for 1 min and they were able to reduce the initial TMAC and YM count below 1 log CFU/mL (Elez-Martinez et al., 2006). Thermal pasteurization at 72 °C for 71 s was able to reduce the natural flora of lemon melon juice blend below detection limits (Kaya, Yildiz, & Unluturk, 2015). Timmermans et al. (2011), on the other hand, equivalently processed orange juice by thermal processing (72 °C for 20 s), HPP and PEF, all treated samples showed microbiological loads below detection limits (Timmermans et al., 2011).

6.3.2. Physicochemical Properties of SJ

Total soluble solid (TSS) content, pH, titratable acidity, and electrical conductivity of treated and untreated strawberry juice samples are provided in Table 6.1. TSS content of control sample was 7.85 ± 0.07 °Brix. No significant difference was observed between control and treated SJ samples. Thermally pasteurized, thermosonicated, HPP and PEF treated SJ samples showed TSS of 7.88 ± 0.04, 8.00 ± 0.14, 7.83 ± 0.04, 7.83 ± 0.04 °Brix, respectively. TSS of fruit juices may vary depending on the maturation of the fruit as well as the fruit juice preparation protocol. Mosqueda-Melgar et al. (2012) reported soluble content of untreated SJ as 7.0 ± 0.01 °Brix. The authors did not observe any difference in TSS content of the strawberry juice subjected to high intensity PEF. On the contrary, they detected significant change in TSS of SJ (7.4 ± 0.2 °Brix) after thermal processing (Mosqueda-Melgar et al., 2012). On the other hand, Odriozola-Serrano et al. (2008) reported soluble solid content of untreated SJ used as 7.2 ± 0.2 °Brix which was in line with the findings of this study. They treated the strawberry juice with PEF using bipolar square-wave pulses of 4 μs at a frequency of 100 Hz and 35 kV/cm field strength for 1,700 μs. SJ was also subjected to the thermal treatment at 90 °C for 30 s and 60 s. They concluded that TSS content of SJ was not significantly changed after subjecting the juice to PEF (Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2008). Thus, the findings of this study were in accordance with the literature.

Untreated SJ had a pH of 3.50 ± 0.01 while the pH of treated samples varied between from 3.45 and to 3.48 (Table 6.1). Even though the pH values of SJ before and after processing seem very close to each other, the sonication process resulted in statistically significant change in pH ($p<0.05$). The observed small differences could be, possibly, attributed to experimental error. This finding is in contrast with the study conducted by Abid et al. (2013). It was reported that no significant change was observed for pH, titratable acidity as well as total soluble solids of apple juice after sonication (Abid et al., 2013). Except sonication, no significant difference was found between control and other pasteurization technologies in aforementioned study. Likewise, Mosqueda-Melgar et al. (2012) reported that pH of SJ (3.26 ± 0.01) was not significantly affected by high intensity PEF and thermal treatments (Mosqueda-Melgar et al., 2012). In the current study, titratable acidity of untreated SJ, on the other hand,

was found as 0.81 ± 0.00 g citric acid per 100 mL. The acidity of treated samples varied from 0.79 to 0.84 g/100 mL. Thermally pasteurized SJ had the lowest acidity (0.79 ± 0.00 g/100 mL) among all samples. Likewise, titratable acidity of strawberry juice used for sonication was reported to be 0.73 g citric acid per 100 mL (Tiwari, O'Donnell, Patras, & Cullen, 2008).

Acidity and pH are important parameters in food processing in order to produce products with consistent well defined properties and to meet the regulatory requirements. Moreover, such parameters affect the consumer acceptability in terms of palatability of final product (Bates, Morris, & Crandall, 2001; Singhal, Kulkarni, & Rege, 1997). It was reported that pasteurization of apple juice by PEF did not affect acidity; thereby one of the important physicochemical property would remain intact with the overall quality of the juice (Aguillar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007). No significant changes were observed for acidity and pH of grapefruit juice subjected to sonication at a frequency of 28 kHz at 20 °C (Aadil, Zeng, Han, & Sun, 2013). Acidity and pH did not show significant differences for HPP treated pomegranate juice after processing at 350, 450 and 550 MPa for 30 s, 90 s and 150 s (Varela-Santos et al., 2012).

Besides, pH has also direct correlation with microbial quality of the product. Since fruit juices have lower pH values, they are highly susceptible to be spoiled by yeasts-molds, lactic acid bacteria, and acid tolerant bacteria (Mosqueda-Melgar et al., 2012). In the case of PEF processing, for instance, microbial spoilage of fruit juices can be delayed as a consequence of formation of pores in the cell membrane of microorganisms due to establishment of the transmembrane potential between the cell membrane and induced electrical field (Coster & Zimmermann, 1975). Once the pores are formed in the cell membrane, undissociated acid molecules tend to dissociate into charged anions in the cell cytoplasm, and gradually decreases intracellular pH. Thus, cell damage occurs in cell signaling, active transport, and genetic material (Raybaudi-Massilia, Mosqueda-Melgar, Soliva-Fortuny, & Martin-Belloso, 2009).

Electrical conductivities of SJ samples varied between 4.05 and 4.11 mS/cm (Table 6.1). There was no significant difference observed in electrical conductivity of SJ before and after processing ($p>0.05$). Electrical conductivity is a critical and essential parameter for the design of a pulse electric field processing. It has been reported to be increased as the temperature escalated (Palaniappan & Sastry, 1991). Altuntas et al. (2010) reported the electrical conductivity of sour cherry juice as 3.64 ± 0.17 mS/cm

(Altuntas, Evrendilek, Sangun, & Zhang, 2010). The strawberry juice was reported to have electrical conductivity of 3.8 mS/cm (Odriozola-Serrano et al., 2008). As a result, the electrical conductivity values of SJ used in this work were in good agreement with those studies cited in the literature.

Table 6.1. Physicochemical properties of SJ samples before and after processing

Process	TSS (°Brix)	pH	Titratable acidity (g/100 mL)	Conductivity (mS/cm)
C	7.85 ± 0.07a	3.50 ± 0.01a	0.81 ± 0.00ab	4.09 ± 0.01a
T	7.88 ± 0.04a	3.48 ± 0.02ab	0.79 ± 0.00b	4.11 ± 0.01a
US	8.00 ± 0.14a	3.45 ± 0.01b	0.84 ± 0.02a	4.10 ± 0.00a
HPP	7.83 ± 0.04a	3.46 ± 0.00ab	0.81 ± 0.00ab	4.08 ± 0.01a
PEF	7.83 ± 0.04a	3.48 ± 0.00ab	0.82 ± 0.00ab	4.05 ± 0.05a

(C: Control, T: Thermal, US: Ultrasonication, HPP: High pressure processing, PEF: Pulsed electric fields, TSS: Total soluble solids, TA: Titratable acidity). Different letters in the same column show the significant differences among treatments on a 95 % of confidence interval ($p<0.05$).

6.3.3. Phytochemical Properties of SJ

Bioactive compound content of SJ was studied in terms of total phenolic content (TPC), total anthocyanin content (TAC), and radical scavenging activity (RSA). These phytochemical properties of SJ samples subjected to different processing technologies were investigated and compared in this section.

6.3.3.1. Total Phenolic Content (TPC)

Total phenolic contents of SJ samples are given in Table 6.2 in terms of gallic acid equivalent (mg/100 mL). TPC of all studied samples varied from 132 to 145 mg/100 mL. In the current study, the untreated sample had TPC of 137.81 ± 0.91 mg/100 mL. Varela-Santos et al. (2012) reported the initial phenolic content of pomegranate juice (untreated sample) as 135.034 mg GAE/100 mL (Varela-Santos et al., 2012). Thus, the results of this study were in good agreement with the one cited by Varela-Santos et al. (2012). Odriozola-Serrano et al. (2008), on the other hand, determined the TPC of strawberry juice as 47.3 mg/100 mL (Odriozola-Serrano et al.,

2008). The differences in the content of total phenolics of juices can be attributed to varietal differences, cultivation techniques (Häkkinen & Törrönen, 2000), processing and storage conditions (Spanos & Wrolstad, 1992).

Table 6.2. Phytochemical content of SJ samples before and after processing

Process	**TPC (mg GAE/100 mL)**	**TAC (mg/L)**	**RSA (%)**
C	137.81 ± 0.91^{ab}	153.31 ± 2.57^{b}	33.72 ± 2.74^{AB}
T	132.21 ± 1.65^{b}	166.42 ± 2.42^{ab}	29.94 ± 3.60^{B}
US	137.59 ± 1.93^{ab}	166.97 ± 0.39^{ab}	39.55 ± 1.92^{A}
HPP	143.53 ± 2.80^{a}	176.67 ± 1.73^{a}	39.98 ± 1.84^{A}
PEF	144.97 ± 1.52^{a}	179.21 ± 8.47^{a}	40.25 ± 0.51^{A}

(All measurements were done at least in triplicate. All values were given as mean ± standard deviation. Different letters within the same column are significantly different considering 95 % of confidence interval ($p<0.05$). C: Control, T: Thermal, US: thermosonication, HPP: high pressure processing, PEF: pulsed electric fields)

PEF and HPP (300 MPa for 1 min) treatments resulted in a slight but non-significant ($p>0.05$) increase in total phenolic content compared to untreated SJ. Thus, PEF and HPP treated samples contained the highest amount of phenolics at a concentration of 144.97 ± 1.52 mg/100 mL and 143.53 ± 2.80 mg/100 mL, respectively. Patras et al. (2009) also reported a slight but non-significant increase in TPC of strawberry and blackberry purees after HPP treatment at 400 and 500 MPa (Patras, Brunton, Da Pieve, & Butler, 2009). Likewise, Ferrari et al. (2010) reported that HPP at 400 MPa led to increase in the total polyphenol content of pomegranate juice (Ferrari, Maresca, & Ciccarone, 2010). Varela-Santos et al. (2012) subjected pomegranate juice to different pressure (350, 450, and 550 MPa) and treatment times (30, 90, 150 s). In accordance with this PhD thesis, the authors observed an increase in amount of TPC in juice samples after HPP treatment. The significant increments in TPC of pomegranate juice were found as 1555 and 1591 mg/L when the juice was treated by HPP at 450 MPa for 90 and 150 s, respectively (Varela-Santos et al., 2012). Corrales et al. (2008) indicated increase in total phenolic content of grape by-products following high pressure processing, ultrasonics and pulsed electric fields (Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008). In the present study, pasteurization by HPP and PEF retained the highest amount of total phenolic content in SJ compared to thermal treatment ($p<0.05$) (Table 6.2). The increase in total phenolic content in HPP treated products has

been related to the increased extractability of some of the antioxidant components due to high pressure. Le Chatelier's theory complies that the volume of the system tends to be reduced during HPP. During treatment, the extracting solvent enters into the cell and interacts with the bioactive constituents (Xi et al., 2009). In the case of PEF, improving the phenolic content of the juice could be attributed to the increase in extraction efficiency of intracellular metabolites due to applied electrical field (Knorr, Angersbach, Eshtiaghi, Heinz, & Lee, 2001). On contrary, Caminiti et al. (2011) observed nonsignificant changes in TPC of apple and cranberry juice blends when the juice subjected to high intensity light pulses (3.3 J/cm^2), UV light (5.3 J/cm^2) and their combinations with pulsed electric fields (34 kV/cm, 18 Hz, 93 μs) and manothermosonication (5 bar, 43 °C, 750 W, 20 kHz) (Caminiti et al., 2011). The phenolic contents of apple and cranberry juice treated by any type of treatment were reported to vary between 57 and 61 mg/100 mL (Caminiti et al., 2011) which was lower compared to strawberry juice studied in the present study most likely due to the composition of bioactive compounds in strawberries (Giampieri et al., 2015).

On the other hand, the initial content of total phenolics was slightly reduced by thermal pasteurization (132.21 ± 1.65 mg/100 mL) and thermosonication (137.59 ± 1.93 mg/100 mL). However, no significant difference was observed among heat treated, thermosonicated and untreated juice samples ($p>0.05$) (Table 6.2). The reduction in TPC of heat treated and thermosonicated juice samples were 4.06 and 0.16 %, respectively (Table 6.3). A similar trend was also observed for the thermosonication of watermelon juice by Rawson et al. (2011). The authors considered varying temperature (25-45 °C) and amplitude (24.1-60 μm) and sonication time (2-10 min) at a constant frequency (20 kHz), and concluded that total phenolic content significantly decreased as the amplitude and processing time increased (Rawson et al., 2011b). Golmohamadi et al. (2013) observed that sonication of red raspberry puree for 10 min of sonication considering frequency of 20 and 490 kHz did not show any significant effect on total phenolics although anthocyanin content increased. Thus, authors suggested that anthocyanins may not have contributed to the results of total phenolic content of puree samples (Golmohamadi, Moller, Powers, & Nindo, 2013). Another study indicated enhancement of bioactive compounds in fruit juices after sonication. Bhat et al. (2011) applied sonication to a lime juice at 25 kHz and 20 °C for 30 and 60 min. They observed that the initial content of bioactive compounds in the lime juice increased with

the sonication for 60 min compared to 30 min of treatment (Bhat, Kamaruddin, Min-Tze, & Karim, 2011). Aguilar-Rosas et al. (2007) reported that thermal pasteurization at 90 °C for 30 min (HTST: high temperature-short time) caused considerable amount of loss in the content of total phenolics in apple juice (Aguillar-Rosas et al., 2007). Overall, the findings of this study were in line with the others cited in the literature.

6.3.3.2. Total Anthocyanin Content (TAC)

Total anthocyanin content was expressed in terms of mg pelargonidin-3-glucoside per L as given in Table 6.2. TAC of SJ samples varied from 153 to 179 mg/L. The range of TAC of SJ samples were actually slightly higher than what Teleszko et al. (2016) reported for TAC of cloudy strawberry juices (67.84 to 133.52 mg/L) obtained from different kinds of cultivars. Thus, varietal changes highly affect the composition of bioactive compounds in juices (Teleszko, Nowicka, & Wojdylo, 2016). Two types of anthocyanidin pigments, i.e. pelargonidin (bright red) and cyanidin (dark red), were responsible for the attractive color of strawberries and their products (Gossinger et al., 2009). Pelargonidin-3-glucoside was reported as the predominant anthocyanin in strawberries, usually followed by pelargonidin-3-rutinoside and cyanidin-3-glucoside. These three major anthocyanins constitute more than 95 % of the total anthocyanins in strawberry (da Silva, Escribano-Bailon, Alonso, Rivas-Gonzalo, & Santos-Buelga, 2007). Similarly, Teleszko et al. (2016) stated that pelargonidin-3-glucoside content varied from 72 to 96 % of total anthocyanins with corresponding concentrations from 67.84 to 133.52 mg/L, respectively (Teleszko et al., 2016). However, the stability of the anthocyanins in food product depends on many factors such as pH, processing temperature, molecular structure, light, oxygen, enzymes, presence of other accompanying substances, storage time, and temperature (Teleszko et al., 2016; Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009a).

In this study, HPP (300 MPa for 1 min) and PEF (35 kV/cm, 27 μs, 155 Hz) treatments significantly enhanced the total anthocyanin content compared to untreated samples. The initial content of anthocyanins was increased by 15.24 and 16.90 % after HPP and PEF treatments (Table 6.3). This finding was in good agreement with the studies conducted by Odriozola-Serrano et al. (2009) and Barba et al. (2012). A remarkable increase in anthocyanin content of strawberry juice subjected to high

intensity PEF processing (35 kV/cm, 1000 μs) was reported to be between 83 % and 102 % in terms of relative retention (Odriozola-Serrano et al., 2009). Barba et al. (2012) achieved a significant increase in the content of total anthocyanins of blueberry juice subjected to PEF treatment at 35 kV/cm (Barba et al., 2012). Some other studies in literature also assessed the efficacy of PEF for improving the extraction of anthocyanins from grape pomace and juice (Barba, Brianceau, Turk, Boussetta, & Vorobiev, 2015; Leong, Burritt, & Oey, 2016). In accordance with TPC findings, the reason of increase in TAC after HPP and PEF can be attributed to the extraction effect of these technologies (Corrales et al., 2008; Knorr et al., 2001; Richard, 1992). HPP enables extraction of monomeric anthocyanins by affecting the membranes in vegetative cells. HPP can increase the cell permeability by disrupting salt bridges and hydrophobic bonds in the cell membrane (Rastogi, Angersbach, & Knorr, 2003). Therefore, HPP could make the anthocyanins more accessible (Cao et al., 2011). PEF provides enhancement of anthocyanins due to the cell membrane permeabilization and subsequent release of intracellular compounds (Soliva-Fortuny, Balasa, Knorr, & Martin-Belloso, 2009). Some recent reviews can be found in literature for more comprehensive knowledge about the effect of HPP and PEF on bioactive constituents of different type of fruit juices (Altuner & Tokusoglu, 2013; Leong et al., 2016; Tokusoglu, 2016).

Table 6.3. Changes in TPC, TAC, and RSA of strawberry juice subjected to different treatments

Treatment	**Changes in percentage**		
	TPC	**TAC**	**RSA**
C	0.00	0.00	0.00
T	-4.06	8.55	-11.22
US	-0.16	8.92	17.30
HPP	4.16	15.24	18.56
PEF	5.20	16.90	19.36

Heat treatment (71.7 °C for 15s) and thermosonication at 55 °C slightly increased the content of total anthocyanins in SJ. However, the increment was not statistically significant. Similarly, Herceg et al. (2013) indicated that sonication can have negligible effects on total anthocyanin content of strawberry and blackberry juice (Herceg, Lelas, Jambrak, Vukušić, & Levaj, 2013). Besides, Bhat and Goh (2017)

studied the impact of sonication at frequency of 25 kHz and temperature of 20 °C for 15 and 30 min of sonication time. The authors observed increase in the TAC of strawberry juice after both 15 and 30 min (Bhat & Goh, 2017). Tiwari et al. (2008) subjected strawberry juice to sonication at varying amplitude levels (from 40 to 100 %) and time (2-10 min) at a constant frequency of 20 kHz. The authors reported a slight enhancement of 1-2 % in the anthocyanin content at low amplitude and treatment time. The increment in anthocyanins has been attributed to the extraction of bound anthocyanins from suspended pulp (Tiwari et al., 2008). In this thesis, thermosonication resulted in 8.92 % increase in the anthocyanin content of strawberry juice after 3 min of sonication at 55 °C and 100 % amplitude. On contrary, higher amplitude levels and time greater than 5 min have been reported to be resulted in the degradation of anthocyanins by Tiwari et al. (2008). Maximum decrease in the anthocyanin content of strawberry juice (3.2 %) was observed when the authors applied sonication at the maximum treatment conditions (Tiwari et al., 2008). Degradation of anthocyanins during sonication could be due to the oxidation reactions promoted by the interactions of free radicals formed during processing (Portenlanger & Heusinger, 1992). Sivasankar et al. (2007) related the sonodegradation mechanism to the pyrolysis within cavitation bubbles or nuclei and generation of $^{\cdot}OH$ radicals which subsequently oxidizes the polar organic compounds (Sivasankar, Paunikar, & Moholkar, 2007; Tiwari et al., 2008). Another reaction mechanism could be either the thermolysis inside the bubble or reaction with hydroxyl radicals resulting in formation of oxidation products on the surface of the bubble (Hart & Henglein, 1985; von Sonntag, Mark, Tauber, & Schuchmann, 1999). Dubrovic et al. (2011) applied sonication at different amplitude (60, 90, and 120 μm), time (3, 6, and 9 min) and temperature (25, 40, and 55 °C) considering a central composite design. Likely, one of the combinations of the parameters was equivalent to the sonication conditions used in this PhD thesis. Thus, the authors were able to compare the content of specific anthocyanin compounds in untreated, thermally pasteurized, and sonicated strawberry juice. They reported the concentration of pelargonidin-3-glucoside, pelargonidin-3-rutinoside, cyanidin-3-rutinoside, and cyanidin-3-glucoside in sonicated strawberry juice as 101.68, 7.05, 6.93, and 0.67 mg/kg, respectively, where the sonication was applied considering 120 μm amplitude, 55 °C, and 3 min of treatment time. The initial content of these specific anthocyanins in strawberry juice was reported to be slightly reduced by sonication (0.7-

4.4 %). However, the degradation caused by sonication was not as intensive as thermal pasteurization (5.3-5.8 %) (Dubrovic, Herceg, Jambrak, Badanjak, & Dragovic-Uzelac, 2011). Thermal processing applies heating at varying temperatures from 50 to 150 °C depending on the type of food product and desired shelf life (Patras, Brunton, O'Donnell, & Tiwari, 2010). Even though inter and intramolecular co-pigmentation with other substances could provide more stability for the anthocyanins towards pH, heat, and light (Francis, 1992), heating has a remarkable influence on anthocyanin stability (Patras et al., 2010). Dubrovic et al. (2011) obtained strawberry juice using seven different cultivars and treated them with thermal and ultrasound processes. They observed that thermal pasteurization at 85 °C for 2 min resulted in the lowest levels of anthocyanins in cloudy strawberry juices compared to untreated or sonicated samples (Dubrovic et al., 2011). On the other hand, additional blanching step (heating to approximately 50 °C) in fruit juice processing could provide a positive influence on retention of anthocyanins (Patras et al., 2010). In the current study, thermal pasteurization at 71.7 °C for 15 s resulted in a slight but nonsignificant increase in the initial content of anthocyanins in accordance with thermosonication studies. The increments in TAC of thermally pasteurized and sonicated strawberry juice were 8.55 % and 8.92 %, respectively (Table 6.3).

6.3.3.3. Antioxidant Activity (RSA) of SJ

Antioxidant activities of samples are given in Table 6.2 as the radical scavenging activity (RSA) in percentage. Antioxidant activity values calculated based on % inhibition capacity of SJ samples on DPPH free radical. Lower absorbance values of the mixture of sample and DPPH indicated higher radical scavenging activity. Antioxidant activities of SJ samples varied from approximately 30 to 40 %. This is partially similar to the previous findings (Bhat & Goh, 2017). The initial antioxidant activity was 33.72 ± 2.74 %. In accordance with TPC results, antioxidant activity of the juice samples significantly increased after HPP and PEF treatments by 18.56 and 19.36 %, respectively, compared to thermal pasteurization (Table 6.3). Hence, the radical scavenging activities of HPP and PEF treated samples were 39.98 ± 1.84 % and 40.25 ± 0.51 %, respectively. The enhancement of the antioxidant activities in HPP and PEF treated SJ samples could be associated with the increased content of total phenolics by

HPP and PEF. Thus, the impact of HPP and PEF technologies on the extraction could result in a product which is richer in antioxidant activity (Knorr et al., 2001; Patras et al., 2009). Alterations in food matrix due to processing could lead to release of compounds having free radical binding attributes (Tadapaneni et al., 2012). Figure 6.2 depicts all the phytochemical properties of SJ subjected to different processes. It gives a good indication how bioactive compounds of strawberry juice retained after application of different processing technologies. It was observed that antioxidant activities of SJ samples almost followed the similar trend with the TPC and TAC. This could be attributed to the contribution of phenolic compounds to the antioxidant activity of the fruit juices (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000; Miller & RiceEvans, 1997).

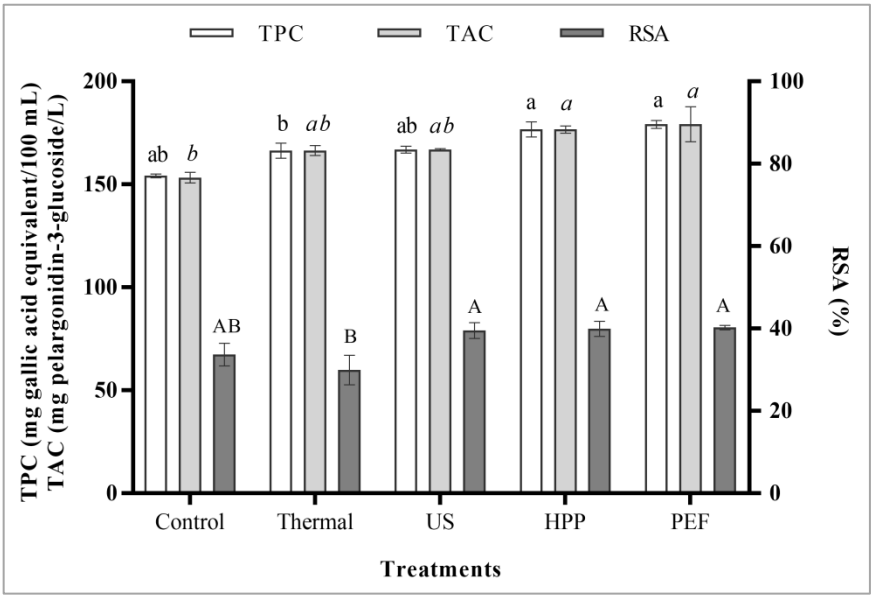


Figure 6.2. Change in TPC, TAC and RSA of strawberry juice samples before and after processing

(Control refers to untreated SJ while thermal, US, HPP, and PEF correspond to thermal pasteurization, thermosonication, high pressure processing, pulsed electric fields. Different letters in the same type of plot shows significant differences among treatments. Small case letters address total phenolic content (TPC), *italic* small case letters indicate differences in terms of total anthocyanin content (TAC), and capital letters refers to the differences among radical scavenging activity (RSA) of SJ samples.)

Bhat and Goh reported that sonication (at 25 kHz frequency with a power set of 70 %) for 15 min and 30 min increased the initial radical scavenging activity (30.42 %) to 31.78 % (non-significant) and 33.61 % (significant), respectively (Bhat & Goh, 2017). Patras et al. (2009) found out that HPP and PEF were able to significantly retain higher antioxidant activities in strawberry and blackberry puree compared to thermal

pasteurization (Patras et al., 2009). These findings were in accordance with the ones obtained in this PhD work. Sanchez-Moreno et al. (2005) stated that HPP and PEF did not alter the antioxidant activity of orange juice; however, thermal pasteurization at high temperature (90 °C for 1 min) was reported to cause a significant decrease in antioxidant activity by 6.56 % (Sanchez-Moreno et al., 2005). In the current study, even though heat treated juice samples showed significantly lower antioxidant activity than thermosonicated, HPP and PEF treated samples; it is a noticeable outcome to indicate that thermal pasteurization at 71.7 °C for 15 s was able to maintain the initial antioxidant activity of untreated SJ with a non-significant difference. Thus, extreme temperatures such as 90 °C, which has been applied as pasteurization temperature (Nagy, Chen, & Shaw, 1993), can be avoided in cases where the practitioner needs to apply thermal treatment to preserve the quality of juice.

6.3.4. Principal Component Analysis

Principal Component Analysis (PCA) reduced the dimension of data matrix, thus enabled a simultaneous evaluation of all physicochemical and phytochemical properties of SJ. Figure 6.3 shows the distribution of treatments in space considering first and second principal components. The first principal component distinctively separated nonthermal pasteurization technologies than the rest. While HPP and PEF treatments were located close to each other, untreated and thermally pasteurized SJ samples fell close to each other. Thermosonication resulted in similar results to HPP and PEF rather than thermal pasteurization.

The locations of the untreated and treated samples were actually based on the physicochemical and phytochemical properties of the juice. The distribution of quality attributes of SJ in space is presented in Figure 6.4. When Figure 6.3 and Figure 6.4 were evaluated together, it can be clearly seen that HPP and PEF treated SJ samples located far from the control sample since they contained higher amount of total phenolics, total anthocyanins and showed higher antioxidant activity compared to control and heat treated SJ. Thus, it could be inferred that HPP and PEF treatments were able to enhance the phytochemical properties of SJ. Since physicochemical properties of SJ before and after treatments were relatively close to each other, the dominating effect in the

principal component analysis was the phytochemical properties which led to a clear separation of samples.

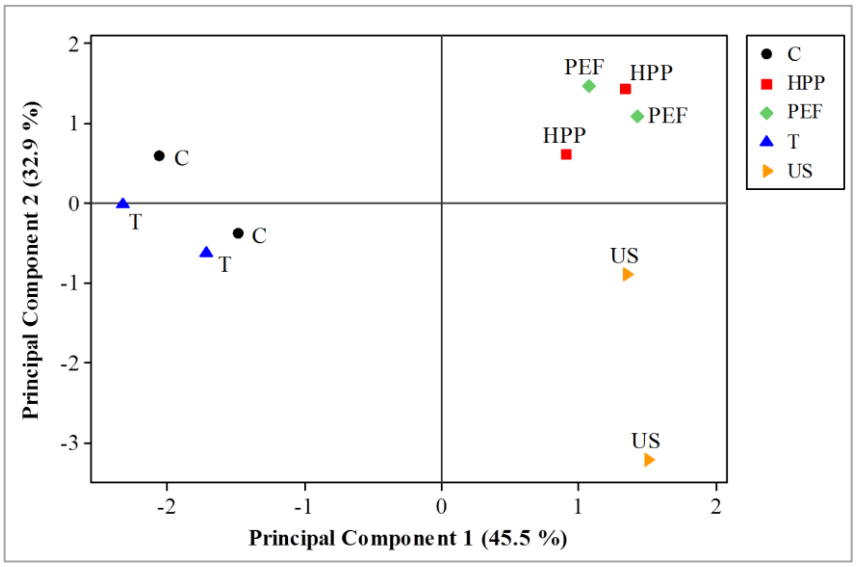


Figure 6.3. Principal component analysis output: Score plot

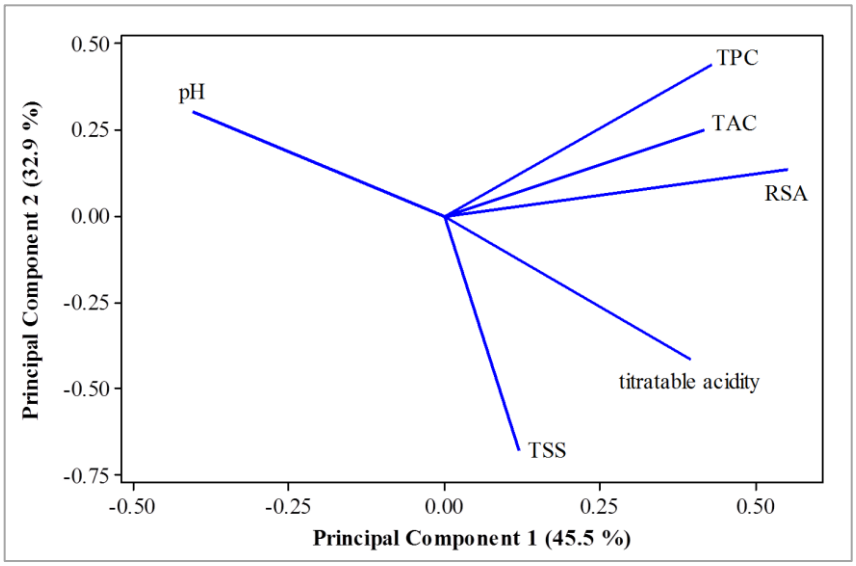


Figure 6.4. Principal component analysis output: Loading plot

The principal component and factor loading values for physicochemical and phytochemical properties of strawberry juice subjected to different treatments are listed in Table 6.4. Phytochemical properties, i.e. TPC, TAC, and antioxidant activity had absolute values of more than 0.41 in the 1st principal component (PC). pH and titratable

acidity also had large absolute values. However, TSS made the lowest contribution (0.121) to the 1st PC. With respect to 2nd PC, the loadings had a different pattern. TSS, titratable acidity, and pH gained greater absolute values, hence physicochemical properties made larger contribution to the 2nd PC in order to distinguish untreated and treated SJ samples.

Table 6.4. Loadings and eigen analysis of principal components

Factor loadings	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**
TSS (°brix)	0.121	-0.680	0.000	0.214	-0.095
pH	-0.404	0.304	0.561	0.572	-0.279
Titratable acidity	0.396	-0.414	0.476	0.302	0.249
TPC	0.430	0.440	0.292	-0.065	0.566
TAC	0.419	0.249	-0.552	0.665	-0.112
DPPH	0.551	0.136	0.261	-0.299	-0.720
Eigen analysis	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**
Eigen value	2.731	1.977	0.773	0.362	0.093
Proportion of variance	0.455	0.329	0.129	0.060	0.015
Cumulative variance	0.455	0.785	0.914	0.974	0.989
% Cumulative variance	45.50	78.50	91.40	97.40	98.90

The PC 1 explained 45.5 % of the total variance in the data set, and PC 2 explained that of 32.9 %. Thus, PCA revealed that untreated and treated SJ samples by different pasteurization methods could be differentiated by phytochemical properties for PC 1, and physicochemical properties for PC 2 considering an explanation of 78.5 % of total cumulative variance. Principal component analysis has been successfully applied for the estimation of correlations among quality parameters (L*, a*/b*, BI, HMF, viscosity, PME and PG) of strawberry juice by Aguilo-Aguayo et al. (2009). The authors were able to explain 99.04 % of the total variance considering the first two principal components (Aguilo-Aguayo, Oms-Oliu, Soliva-Fortuny, & Martin-Belloso, 2009). By means of PCA, Kaya et al. (2015) classified lemon-melon juice blends subjected to conventional thermal pasteurization and UV-C light combined with mild heating and subsequent storage up to 30 days. The authors were clearly distinguished untreated and treated blends during storage in association with the physicochemical properties such as pH, acidity, TSS, turbidity, and color parameters. Likewise, they used the first two principal components which explained 51.09 % of the total variance. Even though the cumulative variance is relatively low compared to the present study, it was still clear enough for the discrimination of samples (Kaya et al., 2015).

6.3.5. Hierarchical Cluster Analysis

A dendrogram was constructed as an output of Hierarchical Cluster Analysis (HCA) in order to describe the similarities and differences among treatments. The plot obtained on the basis of Ward's linkage method and Euclidean distance is given in Figure 6.5. In accordance with PCA, HPP and PEF treated samples associated with each other. Then, thermosonicated samples located close to the cluster of HPP and PEF treatments. On the other hand, untreated and heat treated SJ samples fell close to each other. Classification of the samples correlated to the associated treatments in clusters. Since nonthermal pasteurization technologies enhanced the physicochemical content of SJ samples, they located distant from the control and thermally pasteurized samples. Kaya et al. (2015) well applied HCA for the differentiation of UV-C light treated, heat treated and untreated lemon-melon juice blends during 30 days of refrigerated storage (Kaya et al., 2015).

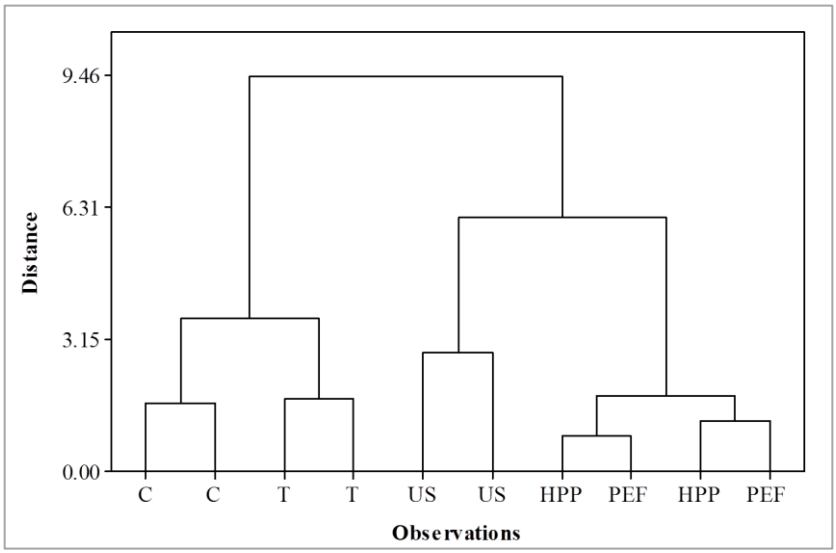


Figure 6.5. Dendrogram for the similarities and differences among treated and untreated juice samples

6.4. Conclusions

In this chapter, processing technologies, i.e. HPP, PEF, thermosonication, and mild thermal treatment, were applied for the pasteurization of freshly squeezed strawberry juice. The overall quality attributes and bioactive compounds retention of SJ were comparatively evaluated in terms of physicochemical (pH, titratable acidity, total

soluble solids) and phytochemical properties (total phenolic content, total anthocyanin content, antioxidant or radical scavenging activity) of SJ samples. The pasteurization conditions determined previously in Chapter 5 on the basis of equivalent microbial inactivation was directly applied to the juice. Hence, SJ was pasteurized by HPP at 300 MPa for 1 min at approximately 20 °C. Thermosonication was performed considering 5.15 ± 1.34 J/mL of acoustic energy, 100 % amplitude (120 μm) in continuous pulsing mode at 55 °C for 3 min of treatment time. The processing conditions for PEF were 35 kV/cm of electric field intensity, 27 μs of treatment time, 155 Hz of frequency, 350 mL/min of flow rate, 2 μs of pulse width in monopolar mode. Thermal pasteurization, on the other hand, was conducted at 71.7 °C for 15 s as mild thermal pasteurization.

After processing, the initial load of natural flora of SJ in terms of TMAC and YM was reduced below 2 log CFU/mL by all technologies. Since microbial safety of the final product is the first prerequisite, this result is a promising output suggesting that nonthermal pasteurization at mild conditions were able to reduce the initial microbial population to an acceptable level. However, different combinations of such technologies can be taken into consideration to increase the lethal efficacy and decrease the initial microbial load below 1 log CFU/mL.

Regarding physicochemical properties, no significant was observed for the total soluble content and electrical conductivity of SJ samples. Some significant changes were detected for pH and titratable acidity of SJ samples; however the differences were numerically small. Bioactive constituents of SJ were well retained by nonthermal technologies. Based on the ANOVA test, it can be concluded that phytochemical contents of HPP and PEF treated SJ samples were significantly greater than that of thermally pasteurized juice. PCA and HCA also showed that HPP and PEF treated samples showed similar properties. The similarities between these products mostly results from the increase in the content of phytochemicals. On the other hand, thermosonication and thermal pasteurization resulted in similar products in terms of physicochemical and phytochemical attributes. Thus, HPP, thermosonication, PEF, and mild thermal pasteurization were well applied for the pasteurization of SJ. Next chapter focused on the changes in physicochemical and phytochemical properties of SJ during refrigerated storage period.

CHAPTER 7

SHELF LIFE OF FRESHLY SQUEEZED STRAWBERRY JUICE TREATED WITH THERMAL AND NONTHERMAL PROCESSES

7.1. Introduction

Fruit juices constitute an important place in the human diet due to their nutritional and health related characteristics. Consumers recently prefer fruit juices with fresh-like properties, convenience, reasonable cost, high nutritional and functional quality, and prolonged shelf life (Sanchez-Moreno, De Ancos, Plaza, Elez-Martinez, & Cano, 2009). Nonthermal processing operations have been revealed as alternative technologies to preserve the nutritional and functional properties as well as to extend the shelf life of the final product. Many studies in literature have contributed to the shelf life extension of fruit juices by high pressure processing (HPP), sonication (US), and pulsed electric fields (PEF). In this regard, HPP has been well applied for mango juice (Santhirasegaram, Razali, George, & Somasundram, 2015b), strawberry juice (Cao, Liu, Wu, Liao, & Hu, 2014), strawberry pulp (Cao et al., 2011), red grapefruit juice (Gao et al., 2015), pomegranate juice (Varela-Santos et al., 2012). Sonication has been claimed as another alternative technology to retard the microbial spoilage and preserve the quality attributes of different types of fruit juices (Guerrouj, Sanchez-Rubio, Taboada-Rodriguez, Cava-Rolla, & Marin-Iniesta, 2016; Martinez-Flores, Garnica-Romo, Bermudez-Aguirre, Pokhrel, & Barbosa-Canovas, 2015). PEF, on the other hand, has been reported to extend the shelf life and retain the quality properties without causing any adverse alterations in several products such as orange juice (Agcam, Akyildiz, & Evrendilek, 2016; Plaza et al., 2006), pomegranate juice (Guo et al., 2014), peach nectar (Altuntas, Evrendilek, Sangun, & Zhang, 2011) compared to thermal pasteurization. Changes in quality attributes of juices immediately after processing by different nonthermal technologies as well as during storage period have been evaluated by some scientists considering equivalent processing approach (Timmermans et al., 2011;

Vervoort et al., 2011; Zulueta, Barba, Esteve, & Frigola, 2013). In this chapter, the microbial stability and retention of physicochemical and phytochemical properties (bioactive compounds) of freshly squeezed SJ treated with HPP, thermosonication, PEF, and conventional thermal pasteurization employed on the basis of equivalent processing approach was monitored during 42 days of refrigerated storage at 4 °C.

7.2. Material and Method

7.2.1. Pasteurization and Storage

The fresh strawberry juice prepared as previously described in Chapter 4 (section 4.1.1). Then the freshly squeezed strawberry juice was subjected to high pressure, thermosonication, PEF and thermal pasteurization under equivalent processing conditions followed by a refrigerated storage at 4 °C. Thermal and nonthermal pasteurization of fresh strawberry juice was conducted as it was explained in Chapter 6 (section 6.2.1). Briefly, SJ was subjected to HPP at 300 MPa for 1 min at approximately 20 °C. Thermosonication was carried out at 55 °C for 3 min considering the maximum amplitude (120 μm) in continuous pulsing mode. PEF processing was conducted at 35 kV/cm of electrical field intensity, 27 μs of treatment time, 155 Hz of frequency, 350 mL/min of flow rate, 2 μs of pulse width in monopolar mode. On the other hand, SJ was thermally pasteurized at 71.7 °C for 15 s. Untreated, HPP, PEF, US and heat-treated strawberry juice samples were then stored in the cold storage room of Washington State University at refrigerated conditions (4 °C) for 42 days. Total mesophilic aerobic bacteria (TMAC), yeasts and mold (YM) counts, physicochemical properties (pH, total soluble content (TSS, °Brix), titratable acidity) and phytochemical properties (total phenolic content (TPC), total anthocyanin content (TAC), and antioxidant (radical scavenging) activity (RSA)) were measured during the storage period. The analytical assays used for determination of physicochemical and phytochemical properties of SJ were outlined in section 4.2 and section 4.3 in Chapter 4. Control samples were analyzed at 0-3-5-7-14th days of storage. On the other hand, processed samples were analyzed weekly to determine the microbial quality and the quantitative changes in physicochemical and phytochemical properties of SJ through storage period, i.e., 42 days. Besides, similarities and differences among treatments were determined with

respect to the physicochemical and phytochemical properties of SJ samples during storage. Additionally, phytochemical properties such as total phenolic content (TPC), total anthocyanin content (TAC), and antioxidant (radical scavenging) activity (RSA) of the samples were monitored during storage, and expressed as mg GAE/100 mL, mg pelargonidin-3-glucoside/L, and %, respectively. More details about the procedures can be found in Chapter 4 and Chapter 6.

7.2.2. Data Analysis

Microbiological quality and physicochemical properties of SJ samples were analyzed in duplicate. Phytochemical assays were conducted in triplicate. All data were analyzed by using Excel worksheet (Microsoft Office 2010, USA) and Minitab 16 software (Minitab Inc., State College, PA, USA) as described in the section 6.2.3. Variance analysis (ANOVA) followed by Tukey comparison test considering 95 % of confidence interval was performed in order to determine the differences among treatments and storage days. Moreover, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to visualize the data structure and distinguish similarities and differences among treatments and storage days considering physicochemical and phytochemical attributes of SJ simultaneously. The details of PCA and HCA are given in Chapter 6, section 6.2.3.

7.3. Results and Discussion

7.3.1. Microbial Quality of Strawberry Juice during Storage

Logarithmic changes in total mesophilic aerobic bacteria (TMAC), yeast and mold (YM) counts of untreated (control), HPP, PEF, US and heat-treated strawberry juice (SJ) during 42 days of refrigerated storage (4.0 °C) were evaluated. Initially, untreated strawberry juice had 3.11 ± 0.12 log CFU/mL of TMAC and 3.41 ± 0.05 log CFU/mL of YM counts, respectively. Figure 7.1 shows the effects of treatments and storage on total mesophilic aerobic bacteria count of SJ. TMAC counts of untreated (control) SJ increased to 4.01 ± 0.05 log CFU/mL within 14 days. The increase in the

number of microorganisms in untreated fruit juice attributed to the naturally occurring microorganisms causing spoilage of fruit juices during refrigerated storage (Vegara, Marti, Mena, Saura, & Valero, 2013).

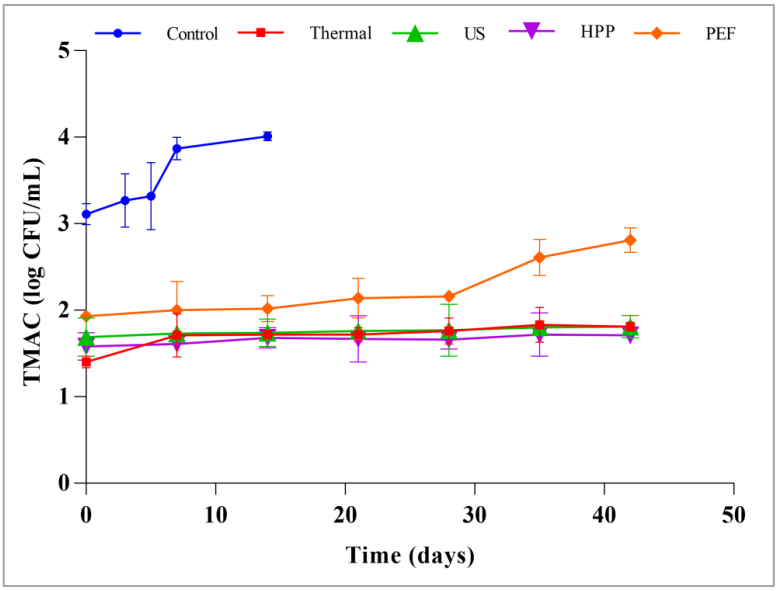


Figure 7.1. The effect of treatments and storage on total mesophilic aerobic bacteria count (TMAC) of strawberry juice
(Control: untreated, Thermal: thermal pasteurization at 71.7 °C for 15s, US: thermosonication, HPP: high pressure processing, PEF: pulsed electric fields)

Figure 7.2, on the other hand, illustrates the effect of treatments and storage on yeast and mold (YM) count of strawberry juice (SJ). YM count of untreated SJ reached to 5.62 ± 0.02 CFU/mL at 14th day of storage period. Molds and yeasts were reported to be the main microorganisms limiting the shelf life of unprocessed SJ (Mosqueda-Melgar, Raybaudi-Massilia, & Martin-Belloso, 2012). According to the microbial criteria mandated by Turkish Food Codex (2002) (Microbiological Criteria, No: 2001/19)) (Codex, 2001) and practical working guidance provided by Institute of Food Science and Technology (IFST, 1999), the acceptable maximum TMAC and YMC counts in fruit juice and nectars must be 4 and 3 log CFU/mL, respectively. Therefore, juice having TAB and YM counts higher than these values is spoiled and cannot be sold in the market (Kaya, Yildiz, & Unluturk, 2015). These limits were taken into consideration as microbial criteria for the shelf life evaluation of SJ pasteurized by different technologies. Thermal and nonthermal pasteurization processes were able to retard the microbial growth (Figure 7.1 and Figure 7.2). Thermal pasteurization at 71.7 °C for 15 s, thermosonication at 55 °C for 3 min, and HPP at 300 MPa for 1 min were

able to keep the TMAC of SJ below 2 log CFU/mL during 42 days of storage period at 4 °C. The maximum counts of total mesophilic aerobic bacteria in SJ treated by heat, thermosonication, and HPP at the end of storage period were found to be 1.81 ± 0.05, 1.81 ± 0.13, and 1.71 ± 0.03 log CFU/mL, respectively, which were below the allowed limit. On the other hand, YM counts of heat treated, thermosonicated, and HPP treated SJ samples at the end of storage were recorded as 1.91 ± 0.13, 1.93 ± 0.04, and 1.97 ± 0.06 log CFU/mL, respectively. Since the microbial counts in terms of TMAC and YM were below the microbial criteria, it was concluded that selected nonthermal and thermal processes were able to extend the shelf life of SJ up to 42 days under refrigerated conditions. TMAC count of PEF-treated juice reached to 2.81 ± 0.14 CFU/mL at the end of storage period (42 days). However YM count of PEF treated SJ was recorded as 2.60 ± 0.03 log CFU/mL at the end of 28th day. YM count of SJ gradually increased after the 28th day reaching to 3.21 ± 0.37 log CFU/mL at 35th day and 4.04 ± 1.38 log CFU/mL at the end of storage period (42nd day). When the microbial criteria of Turkish Food Codex were taken into consideration, PEF-treated juice was assumed to be spoiled at 35th day of storage. It was concluded that thermal pasteurization, thermosonication, and HPP processes successfully extended the shelf life of SJ up to 42 days; whereas PEF processing prolonged the shelf life of SJ up to at least 28th days at refrigerated conditions.

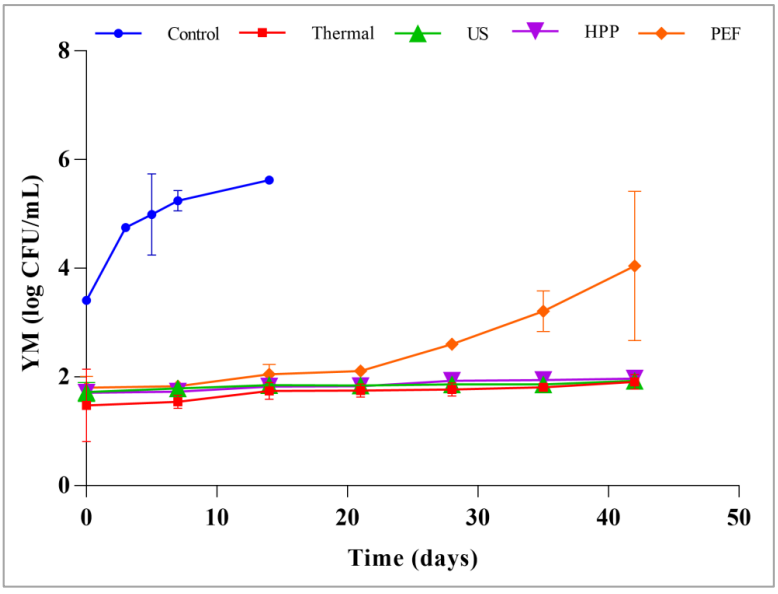


Figure 7.2. The effect of treatments and storage on yeast and mold (YM) count of strawberry juice (SJ)
(Control: untreated, Thermal: thermal pasteurization at 71.7 °C for 15s, US: thermosonication, HPP: high pressure processing, PEF: pulsed electric fields)

The results of this study were in good agreement with the findings of others cited in the literature. For example, the shelf life of mango juice was extended at least 4 weeks by sonication applied for 30 min compared to control sample (Santhirasegaram et al., 2015b). However, the sonication time used by Santhirasegaram et al. (2015b) was longer than the one used in the current study. In this study, the sonication was combined with a moderate temperature which resulted in shortened processing time of SJ. Similarly, Martínez-Flores et al. (2015) processed carrot juice by sonication (24 kHz, 120 μm amplitude) at three different temperatures (50 °C, 54 °C and 58 °C) for 10 min. The authors were also able to extend the shelf life of carrot juice up to 20 days at 4 °C. However, the microbial growth during storage of carrot juice was higher compared to the findings of this study. As stated by the authors, carrot juice sonicated at 58 °C had 3 log CFU/mL of total mesophilic aerobic count, and 4.5 log CFU/mL of yeast and molds count at the end of 20 days of storage period (Martinez-Flores et al., 2015). In the current study, on the other hand, the shelf life of SJ was extended to 42 days by sonication (24 kHz, 120 μm amplitude) at 55 °C for 3 min. The reason of achieving longer shelf life for strawberry juice when compared with carrot juice can be attributed to the lower pH value of SJ. Many studies related to sonication have been primarily concentrated on the changes in quality parameters of several fruit juices (Abid et al., 2014a; Bhat & Goh, 2017; Bhat, Kamaruddin, Min-Tze, & Karim, 2011; Rawson et al., 2011b) rather than microbial stability of juices during storage. This can be aroused from the fact that sonication is more effective to accomplish the required amount of microbial reduction when it is combined with other technologies (Piyasena, Mohareb, & McKellar, 2003). The microbial inactivation by sonication has been attributed to the creating high pressure shock waves, thinning and disrupting of cell membranes, localized heating and production of free radicals (Butz & Tauscher, 2002; Piyasena et al., 2003). In order to increase the lethality of the treatment, sonication has been tended to be combined with other thermal or nonthermal processing technologies such as PEF (Walkling-Ribeiro, Noci, Cronin, Lyng, & Morgan, 2009), pressure (Abid et al., 2014b), antimicrobials (Munoz et al., 2012). It has been claimed that the additive or synergistic effect obtained from the combination of sonication and moderate heat (thermosonication) could improve the lethal effect of ultrasound (Lee, Zhou, Liang, Feng, & Martin, 2009). However, there are limited studies related to the shelf life extension of fruit juices by thermosonication. Thus, this study revealed the potential of

thermosonication to be used as an alternative pasteurization method for extending the shelf life of strawberry juice.

The total mesophilic aerobic bacteria (TMAC) count of HPP processed juice samples was also found to be below 2 log CFU/mL throughout the whole storage period. The highest TMAC of HPP treated SJ was recorded as 1.71 ± 0.03 log CFU/mL at the end of 42nd day of refrigerated storage (Figure 7.1). Even though limited information is available in literature about the microbial stability and safety of HPP treated fruit juices stored for extended periods, this study showed a good correlation with the previous research findings. Varela-Santos et al. (2012) also reported that HPP at 350 MPa for 150 s was able to extend the microbiological shelf life of pomegranate juice by more than 35 days under refrigerated conditions (Varela-Santos et al., 2012). Bull et al. (2004) applied HPP to orange juice at 600 MPa for 60 s. Accordingly, the authors were able to reduce the microbial load of orange juice to non-detectable levels. The microbial growth in orange juice was kept below the detection limit during the first 4 weeks of refrigerated storage (4 °C) and then it was stabilized around 2 log CFU/mL throughout 12 weeks of storage (Bull et al., 2004). Briones et al. (2010) pointed out that refrigerated storage has influence on the delay of the onset of the microbial growth phase (Briones, Reyes, Tabilo-Munizaga, & Perez-Won, 2010). Accordingly, Patterson et al. (2012) were able to reduce the initial microbial load to approximately 1.7 log CFU/mL after subjecting the carrot juice to HPP at 500 and 600 MPa for 1 min (20 °C). The authors then observed very little increase in the survival number of microorganisms of HPP treated carrot juice during 22 days of refrigerated storage (4 °C). However, storage of HPP treated carrot juice at 12 °C caused an increase in the microbial count reaching to 7 log CFU/mL by the end of day 10 whereas storage at 4 °C were able to keep the microbial count below 3 log CFU/mL until the end of storage period (22 days) (Patterson, Mckay, Connolly, & Linton, 2012). On the other hand, this study showed that the microbial shelf life of HPP treated strawberry juice was 42 days which was longer than the shelf life of HPP treated carrot juice. The differences in the shelf life of HPP treated SJ and carrot juice could be attributed to pH of these products. The carrot juice possess higher pH values (pH 6.2) (Teo, Ravishankar, & Sizer, 2001) compared to that of strawberry juice (pH 3.9) used in this study. This is because lower pH values would contribute to the destruction of microorganism in combination with HPP (Tola & Ramaswamy, 2014). Krebbers et al. (2003) reported that HPP treatment up to 500 MPa

at ambient temperature caused a moderate inactivation in the natural microbial flora of tomato puree. HPP at 700 MPa and 20 °C reduced the number of natural microflora of tomato puree below detection limits (Krebbers et al., 2003). Cao et al. (2012) extended the shelf life of turbid and clear strawberry juices up to 6 months by subjecting the juices to 600 MPa for 4 min and subsequent storage at 4 °C (Cao et al., 2012). Besides, Dede et al. (2007) reported that the combination of pressure with mild heat could enhance the lethal effect of the treatment. HPP of carrot juice at 250 MPa for 15 min at 35 °C was able to reduce the total aerobic count below the detection limit (Dede, Alpas, & Bayindirli, 2007). Similarly, Tonello (2011) indicated that the higher pressures and shorter processing times would likely to be more desirable from commercial point of view (Tonello, 2011). Consequently, the findings of this study are in a good line with the previous researches.

The PEF treatment of SJ at 35 kV/cm of electrical field intensity, 27 μs of treatment time, and 155 Hz of frequency was able to keep the total aerobic bacteria counts around 2 log CFU/mL throughout 28 days of storage. A remarkable increase in the TMAC and YM counts were observed at 35^{th} day and 42^{nd} day of storage (Figure 7.1). The final TMAC of PEF treated SJ increased to 2.81 ± 0.14 CFU/mL at the end of storage. These findings were partially in agreement with the study conducted by Guo et al. (2014). They treated pomegranate juice with PEF applied at an electrical field intensity of 35 and 38 kV/cm for 281 μs at 55 °C resulted in a significant inhibition of total aerobic bacteria. TMAC was kept below 2.5 log CFU/mL during 12 weeks of storage at 4 °C. They were also able to reduce the YM counts in pomegranate juice below detectable limits after processing. Accordingly, YM count was kept below 3 log CFU/mL up to 12 weeks. Thus it would be concluded that PEF processing was able to extend microbial shelf life of pomegranate juice up to 12 weeks (Guo et al., 2014). Elez-Martínez et al. (2006) were able to reduce the number of naturally present microorganisms below 1 log CFU/mL when the orange juice was processed by high intensity PEF (35 kV/cm for 1,000 μs; bipolar 4-μs pulses at 200 Hz). Moreover, the authors found out that the microbiological shelf life of HIPEF treated orange juice was extended up to 56 days at 4 °C. However, storage at 22 °C resulted in higher microbial counts (>4 log CFU/mL) at the 28^{th} day. Thus, storage at 4 °C is favorable to provide the extension of shelf life of orange juice (Elez-Martinez, Soliva-Fortuny, & Martin-Belloso, 2006). The processing conditions, i.e. number of pulses, treatment time, type of

pulse, etc., applied to orange juice in the study of Elez-Martinez et al. (2006) were more intense compared to the PEF processing conditions applied for strawberry juice in this study. Because of this reason, it is speculated that the shelf life of orange juice was longer than the strawberry juice. Additionally, the influence of storage temperature was previously addressed by Evrendilek et al (2000) concluding that PEF processed apple juice and cider samples had a shelf life more than 67 days at 4°C, approximately 67 days at 22 °C, and 14 days at 37 °C whereas the control samples had a shelf life of approximately 14 days at 4 °C and less than 3 days at 22 and 37 °C, respectively (Evrendilek et al., 2000). Mosqueda-Melgar et al. (2008) applied high intensity PEF (HIPEF) (35 kV/cm at 193 Hz and 4 μs pulse duration) to melon and watermelon juices containing natural antimicrobials (citric acid and cinnamon bark oil). Complete inactivation of mesophilic bacteria, psychrophilic bacteria and yeast and mold were accomplished in both juices subjected to HIPEF treatment. The HIPEF increased the shelf life of both juices by more than 91 days of storage at 5 °C (Mosqueda-Melgar, Raybaudi-Massilia, & Martin-Belloso, 2008).

The storage time (42 days) studied for the strawberry juice pasteurized by nonthermal technologies was relatively shorter than previously published shelf life studies in literature. HPP and US treated SJ samples might show microbial stability at refrigerated storage for extended periods more than 42 days. However, this hypothesis would not apply for PEF treated SJ since the total aerobics, yeast and mold counts reached to the critical limits at 35^{th} day of storage.

Thermal pasteurization (71.7 °C for 15s), on the other hand, was able to ensure the microbial safety of SJ throughout the storage period (42 days). In literature, different time and temperature parameters were applied for shelf life extension of fruit juices. For example, Bull et al (2004) applied relatively low temperature (65 °C for 60 s) heat treatment to Valencia orange juice which had high initial load of aerobic bacteria and yeast-mold. They stated that the thermal treatment was able to reduce the aerobic bacteria population to 4.3 log CFU/mL while the yeast and mold count was reported to be 3 log CFU/mL after thermal pasteurization (Bull et al., 2004). Thus, this study showed that moderate temperatures may not be sufficient when the initial microbial load was quite high. In order to start the microbiological shelf life of the product at the safe microbial levels, thermal treatments at higher temperatures have been also widely applied to food products. Min et al. (2003) pasteurized orange juice at 90 °C for 90 s,

and extended its shelf life by 196 days at 4 °C (Min, Jin, Min, Yeom, & Zhang, 2003). In another study, thermal pasteurization at 90 °C for 1 min achieved microbial counts below 1 log CFU/mL in orange juice and extended the shelf life of the juice up to 56 days (Elez-Martinez et al., 2006). Walkling-Ribeiro et al. (2009) applied High-Temperature Short-Time (HTST) treatment method (94 °C for 26 s) for pasteurization of orange juice, and observed that the microbial counts were below the detection limits during 168 days of storage (Walkling-Ribeiro et al., 2009). In conclusion, shelf life study of strawberry juice was conducted shorter than related studies in literature. Similar to HPP and thermosonicated samples, the microbial load of heat treated SJ was also below critical limits up to 42 days at refrigerated conditions. Therefore, thermally pasteurized product would likely to maintain its microbial stability for more than 42 days.

7.3.2. Physicochemical Properties Changes during Storage of Strawberry Juice

Influence of storage duration on the physicochemical properties, i.e. pH, titratable acidity (g/100 mL), total soluble content (TSS) (°Brix), of SJ samples subjected to different thermal and nonthermal processes are evaluated and quantitative changes are given in Table 7.1. The initial TSS of strawberry juice was 7.85 ± 0.07 °Brix. Similarly, Odriosola Serrrano et al. (2008) reported the range of TSS of both pasteurized strawberry juice by PEF and thermal treatments, and fresh strawberry juice to be between 7.0 and 7.2 °Brix (Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2008). This range was very close to that of SJ in this study. Tiwari et al. (2008) used a strawberry juice containing total soluble solids of 9.61 °Brix for sonication treatment (Tiwari, O'Donnell, Patras, & Cullen, 2008) which was slightly higher than that of juice used in the current study.

Total soluble content of untreated SJ decreased from 7.85 ± 0.07 to 7.70 ± 0.07 °Brix during 14 days of refrigerated storage. Even though the decrease in TSS of untreated SJ was not statistically significant ($p>0.05$), Elez-Martinez et al. (2006) attributed this difference to the consumption of sugars by microorganisms. In other words, the microbial growth in the orange juice was directly correlated with the

consumption of sugars during storage period (Elez-Martinez et al., 2006). On the other hand, after processing of SJ by thermal, thermosonication, HPP, and PEF treatments, no significant changes were observed in the TSS of SJ samples ($p>0.05$) (Table 7.1), This result was in good agreement with the findings of Aadil et al. (2015) who reported similar result for grapefruit juice treated by PEF processing (Aadil et al., 2015). The results of this study was also in line with the studies of equivalently processed orange juice by thermal, HPP, and PEF (Timmermans et al., 2011), and sonicated grapefruit juice (Aadil, Zeng, Han, & Sun, 2013). During storage of treated SJ samples, no significant changes in TSS of SJ samples were observed during 42 days irrespective of treatment (Table 7.1). This finding is in agreement with other studies reported previously for different fruit juices subjected to heat, thermosonication, HPP, and PEF (Elez-Martinez et al., 2006; Timmermans et al., 2011; Walkling-Ribeiro et al., 2009).

Untreated SJ had pH value of 3.50 ±0.01 and titratable acidity of 0.81 ± 0.00 g/100 mL (Table 7.1). The strawberry juice used in the study of Tiwari et al. (2008) was reported to have pH value of 3.14 and acidity of 0.73 g/100 mL (Tiwari et al., 2008) which were similar to the characteristics of SJ used in the present study. The changes in pH and acidity immediately after processing were discussed in Chapter 6. In the literature, it is commonly reported that juice products have showed insignificant change in pH and acidity during storage period (Barba, Esteve, & Frigola, 2012b; Odriozola-Serrano, Aguilo-Aguayo, Soliva-Fortuny, & Martin-Belloso, 2013).

Although the differences were small among the storage days, the results of this study indicated significant change ($p<0.005$) in pH and acidity of treated SJ ranging between 3.39 and 3.51, and 0.79 and 0.88 g/100 mL, respectively, throughout refrigerated storage (Table 7.1). In contrast, Tiwari et al. (2009) observed no significant changes in the titratable acidity and TSS of sonicated orange juice irrespective of amplitude level, treatment time or storage time (Tiwari, Donnell, Muthukumarappan, & Cullen, 2009c). On the other hand, the authors observed some significant changes in pH (Tiwari et al., 2009c) in accordance with the results of this study. Martinez-Flores et al. (2015) attributed the change in pH of sonicated carrot juice during storage to the new chemical compounds generated in the media because of the ultrasound processing (Martinez-Flores et al., 2015).

Table 7.1. Influence of storage duration on the physicochemical properties of SJ subjected to different thermal and nonthermal processes

Time (day)	Treatment	TSS (°Brix)	pH	Titratable acidity (g/100 mL)
0	Control	^{a}7.85 ± 0.07**A**	^{a}3.50 ± 0.01**A**	^{ab}0.81 ± 0.00**A**
	T	^{a}7.88 ± 0.04a	^{ab}3.48 ± 0.02ab	^{b}0.79 ± 0.00b
	US	^{a}8.00 ± 0.14A	^{b}3.45 ± 0.01B	^{a}0.84 ± 0.02A
	HPP	^{a}7.83 ± 0.04*a*	^{ab}3.46 ± 0.00*a*	^{ab}0.81 ± 0.00*c*
	PEF	^{a}7.83 ± 0.04**a**	^{ab}3.48 ± 0.00**a**	^{ab}0.82 ± 0.00**a**
3	Control	7.83 ± 0.04**A**	3.48 ± 0.01**A**	0.82 ± 0.00**A**
5	Control	7.83 ± 0.04**A**	3.47 ± 0.05**A**	0.82 ± 0.00**A**
7	Control	7.80 ± 0.00**A**	3.46 ± 0.04**A**	0.84 ± 0.02**A**
	T	7.98 ± 0.04a	3.51 ± 0.01a	0.82 ± 0.00ab
	US	7.93 ± 0.04A	3.47 ± 0.00A	0.84 ± 0.00A
	HPP	7.90 ± 0.00*a*	3.47 ± 0.01*a*	0.82 ± 0.00*bc*
	PEF	7.90 ± 0.07**a**	3.45 ± 0.00**b**	0.82 ± 0.00a
14	Control	7.70 ± 0.07**A**	3.44 ± 0.00**A**	0.84 ± 0.02**A**
	T	7.95 ± 0.07a	3.46 ± 0.01ab	0.83 ± 0.01ab
	US	7.98 ± 0.04A	3.43 ± 0.00BC	0.85 ± 0.01A
	HPP	7.95 ± 0.07*a*	3.42 ± 0.01*b*	0.85 ± 0.01*ab*
	PEF	7.85 ± 0.07**a**	3.42 ± 0.00**c**	0.84 ± 0.04**a**
21	T	7.85 ± 0.07a	3.45 ± 0.05ab	0.81 ± 0.01ab
	US	7.80 ± 0.00A	3.40 ± 0.00D	0.84 ± 0.02A
	HPP	7.80 ± 0.00*a*	3.41 ± 0.00*bc*	0.86 ± 0.01*a*
	PEF	7.70 ± 0.00**a**	3.40 ± 0.00**d**	0.86 ± 0.00**a**
28	T	7.90 ± 0.00a	3.42 ± 0.00b	0.82 ± 0.00ab
	US	7.88 ± 0.04A	3.40 ± 0.01D	0.84 ± 0.03A
	HPP	7.88 ± 0.04*a*	3.39 ± 0.00*c*	0.86 ± 0.00*a*
	PEF	7.80 ± 0.00**a**	3.39 ± 0.00**d**	0.86 ± 0.02**a**
35	T	7.85 ± 0.07a	3.41 ± 0.00b	0.83 ± 0.00ab
	US	7.80 ± 0.00A	3.41 ± 0.00CD	0.85 ± 0.00A
	HPP	7.80 ± 0.00*a*	3.39 ± 0.00*c*	0.86 ± 0.01*a*
	PEF	7.60 ± 0.14**a**	3.40 ± 0.01**d**	0.86 ± 0.02**a**
42	T	7.85 ± 0.07a	3.47 ± 0.01ab	0.85 ± 0.02a
	US	7.90 ± 0.00A	3.43 ± 0.01B	0.85 ± 0.00A
	HPP	7.85 ± 0.07*a*	3.39 ± 0.00*c*	0.85 ± 0.00*ab*
	PEF	7.58 ± 0.18**a**	3.39 ± 0.00**d**	0.88 ± 0.02**a**

T, US, HPP, PEF refer to thermal pasteurization, thermosonication, high pressure processing, pulsed electric fields, respectively. Results were given as mean ± standard deviation. Different bold upper case, lower case, upper case, italic lower case and bold lower case letters indicate the significant differences during 42 days of storage of untreated SJ and SJ treated by thermal pasteurization, US, HPP, and PEF, respectively. With respect to day 0, different lower case letters given on the left side of the data as superscript show the significant differences among treatments ($p<0.05$).

On the contrary, Bull et al. (2004) stated that pH of Valencia and Navel orange juice subjected to HPP did not significantly change over storage time (Bull et al., 2004). Walkling-Ribeiro et al. (2009) found out no significant differences for both TSS and pH values of orange juice samples subjected to thermosonication (55 °C for 10 min) followed by PEF (40 kV/cm for 150 μs), thermal pasteurization (94 °C for 26 s) and subsequent storage at 25 °C up to 168 days (Walkling-Ribeiro et al., 2009). Similarly, no significant changes in the acidity of thermosonicated and PEF treated SJ were detected during 42 days of storage in the present study.

7.3.3. Phytochemical Properties Changes during Storage of Strawberry Juice

7.3.3.1. Total Phenolic Content

Influence of storage duration on the phytochemical properties of SJ subjected to different thermal and nonthermal processes was evaluated. The quantitative changes in phytochemical properties right after the processing and during storage are shown in Table 7.2. The effect of storage time on total phenolic content (TPC) of SJ samples subjected to different treatments is depicted in Figure 7.3. Untreated SJ had a TPC of 137.81 ± 0.91 mg/100 mL. As discussed thoroughly in Chapter 6, TPCs of HPP and PEF treated SJ were significantly increased after the processing as compared to thermally pasteurized juice samples. The initial TPCs of heat, thermosonication, HPP, and PEF treated samples were 132.21 ± 1.65, 137.59 ± 1.93, 143.53 ± 2.80, 144.97 ± 1.52 mg/100 mL at the beginning of storage period (day 0). A remarkable increase in TPC of HPP and PEF treated SJ was observed at 7^{th} day of refrigerated storage (Figure 7.3). Although, the increase was not significant for PEF treated SJ ($p>0.05$); a significant elevation was observed in TPC of HPP treated juice samples ($p<0.05$) (Table 7.2). Thermal processing resulted in the lowest content of total phenolics in SJ samples at the end of storage followed by thermosonication, PEF, and HPP treatments. In fact, total phenolic content remained higher in HPP and PEF treated SJ samples compared to thermally pasteurized juice throughout storage. This result is in accordance with the study conducted by Plaza et al. (2011). They indicated that HPP and PEF treatments

were more effective for the preservation of bioactive compounds in orange juice in comparison to low temperature pasteurization (70 °C for 30 min) during storage period (Plaza et al., 2011). However, at the end of 42 days of refrigerated storage, TPC of SJ samples did not show significant difference among processing technologies ($p>0.05$).

Table 7.2. Influence of storage duration on the phytochemical properties of SJ subjected to different thermal and nonthermal processes

Time (days)	Sample	TPC (mg/100 mL)	TAC (mg/L)	RSA (%)
0	C	^{ab}137.81 ± 0.91	^{b}153.31 ± 2.57	^{ab}33.72 ± 2.74
	T	^{b}132.21 ± 1.65a	^{ab}166.42 ± 2.42a	^{b}30.00 ± 2.18b
	US	^{ab}137.59 ± 1.93A	^{ab}166.97 ± 0.39A	^{ab}39.55 ± 1.92B
	HPP	^{a}143.53 ± 2.80*b*	^{a}176.67 ± 1.73*a*	^{a}39.98 ± 1.84*bc*
	PEF	^{a}144.97 ± 1.52**ab**	^{a}179.21 ± 8.47**a**	^{a}40.25 ± 0.51**b**
7	T	128.70 ± 19.95a	150.81 ± 2.83ab	50.58 ± 1.10a
	US	138.17 ± 5.15A	155.27 ± 10.59AB	54.17 ± 0.54A
	HPP	161.40 ± 4.08*a*	157.13 ± 3.28*b*	55.77 ± 1.24*a*
	PEF	153.82 ± 20.45**a**	161.21 ± 2.54**abc**	53.96 ± 0.98**a**
14	T	127.15 ± 11.41 a	144.31 ± 5.07bc	48.28 ± 2.34a
	US	131.50 ± 1.15A	154.35 ± 1.58ABC	48.75 ± 0.04A
	HPP	133.25 ± 1.65*bc*	156.92 ± 2.99*b*	49.61 ± 1.17*ab*
	PEF	132.67 ± 5.88**bcd**	159.60 ± 0.26**b**	49.93 ± 3.11**a**
21	T	116.91 ± 1.76a	133.40 ± 1.52bc	31.94 ± 0.89b
	US	122.94 ± 2.74AB	140.23 ± 3.44BCD	38.78 ± 0.14B
	HPP	123.45 ± 2.61*c*	152.37 ± 4.67*b*	39.74 ± 6.42*bc*
	PEF	123.30 ± 0.00**cde**	152.83 ± 0.22**bcd**	35.16 ± 1.14**b**
28	T	102.37 ± 1.74a	127.59 ± 3.73c	37.05 ± 1.97b
	US	115.88 ± 13.43AB	137.19 ± 1.18CD	37.38 ± 3.91B
	HPP	121.39 ± 7.85*cd*	144.30 ± 8.26*b*	36.02 ± 0.78*c*
	PEF	114.84 ± 6.37**de**	142.97 ± 2.92**cd**	35.01 ± 0.46**b**
35	T	102.17 ± 0.27a	125.41 ± 8.73c	34.27 ± 5.10b
	US	105.59 ± 2.54B	136.30 ± 1.60D	38.80 ± 0.37B
	HPP	108.07 ± 0.47*d*	143.26 2.86*b*	38.97 ± 0.95*c*
	PEF	106.86 ± 6.42**e**	141.20 ± 0.98**d**	37.97 ± 0.78**b**
42	T	^{a}101.75 ± 0.25a	^{a}125.30 ± 5.55c	^{a}34.04 ± 1.00b
	US	^{a}104.41 ± 2.71B	^{a}128.34 ± 2.41D	^{a}38.80 ± 0.60B
	HPP	^{a}107.04 ± 0.08*d*	^{a}140.30 ± 2.50*b*	^{a}36.98 ± 0.34*c*
	PEF	^{a}104.48 ± 1.87**e**	^{a}140.96 ± 2.35**d**	^{a}38.48 ± 0.73**b**

T, US, HPP, PEF refer to thermal pasteurization, thermosonication, high pressure processing, pulsed electric field, respectively. Results were given as mean ± standard deviation. Different lower case, upper case, italic lower case and bold lower case letters indicate the significant differences during 42 days of storage of SJ treated by thermal pasteurization, US, HPP, and PEF, respectively. With respect to day 0, different lower case letters given on the left side of the data as superscript show the significant differences among treatments ($p<0.05$).

The content and stability of total polyphenols in juices have been reported to be dependent on duration of storage conditions (Teleszko, Nowicka, & Wojdylo, 2016) as well. Accordingly, a decreasing trend was expected for TPC of all SJ samples during storage period. However, a significant increase was observed at the 7th day of storage. This finding was contrary to the results presented by Barba et al. (2012) who conducted a storage study with HPP and PEF treated blueberry juice. The authors indicated a significant decrease after 7 days of refrigerated storage of blueberry juice subjected to HPP and PEF. Afterwards, the authors also reported that they have noticed some fluctuation such as increase and decrease in the content of total phenolics during 56 days of refrigerated storage at 4 °C (Barba et al., 2012). In this work, TPC of all samples decreased from 7th day to 42nd day irrespective of treatment type (Figure 7.3).

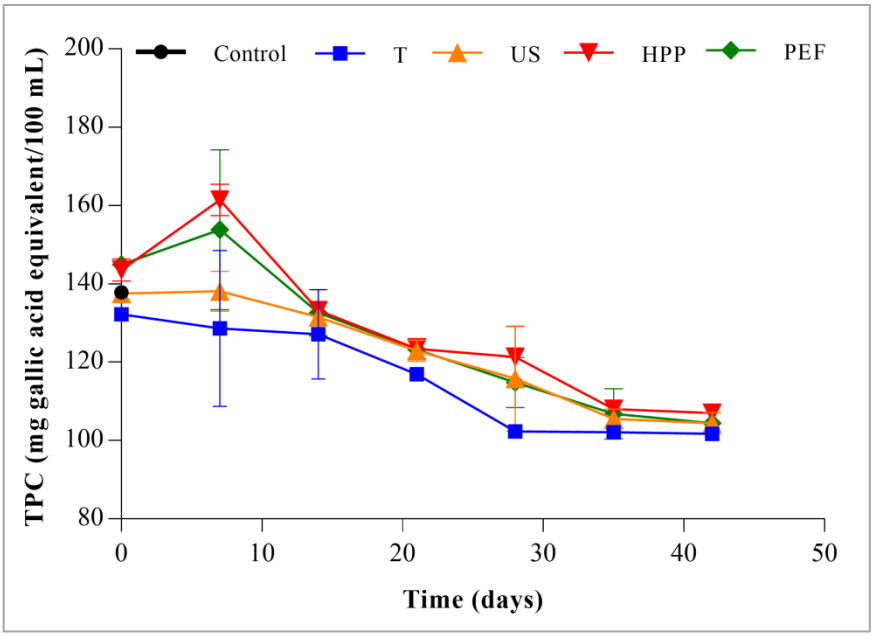


Figure 7.3. The effect of storage time on the Total Phenolic Content (TPC) of strawberry juice subjected to different treatments
(T, US, HPP, PEF refer to thermal pasteurization, thermosonication, high pressure processing, pulsed electric fields, respectively)

Table 7.3 shows the retention of phytochemical properties of SJ samples in terms of percentage throughout the storage. In this table, retention percentages at day 0 were calculated considering untreated SJ as 100 %. On the other hand, the percent retention of phytochemical properties of SJ samples during storage starting from day 7th up to day 42nd was calculated considering the initial contents of the related properties of heat, thermosonication, HPP, and PEF treated SJ samples.

Table 7.3. Percentage Retention of phytochemical properties of SJ processed with different technologies during 42 days of storage at 4 °C

Storage Day	Process	TPC (%)	TAC (%)	RSA (%)
0	C	100^{ab}	100^{b}	100^{a}
	T	96^{b}	109^{ab}	89^{a}
	US	100^{ab}	109^{ab}	117^{a}
	HPP	104^{a}	115^{a}	119^{a}
	PEF	105^{a}	117^{a}	119^{a}
7	T	97^{A}	91^{A}	169^{A}
	US	100^{A}	93^{A}	137^{A}
	HPP	112^{A}	89^{A}	140^{A}
	PEF	106^{A}	90^{A}	134^{A}
14	T	96^{A}	87^{A}	162^{A}
	US	96^{A}	92^{A}	123^{A}
	HPP	93^{A}	89^{A}	124^{A}
	PEF	92^{A}	89^{A}	124^{A}
21	T	88^{A}	80^{A}	107^{A}
	US	89^{A}	84^{A}	98^{A}
	HPP	86^{A}	86^{A}	99^{A}
	PEF	85^{A}	85^{A}	87^{A}
28	T	77^{A}	77^{A}	124^{A}
	US	84^{A}	82^{A}	95^{A}
	HPP	85^{A}	82^{A}	90^{A}
	PEF	79^{A}	80^{A}	87^{A}
35	T	77^{A}	75^{A}	115^{A}
	US	77^{A}	82^{A}	98^{A}
	HPP	75^{A}	81^{A}	98^{A}
	PEF	74^{A}	79^{A}	94^{A}
42	T	77^{A}	75^{A}	114^{A}
	US	76^{A}	77^{A}	98^{A}
	HPP	75^{A}	79^{A}	93^{A}
	PEF	72^{A}	79^{A}	96^{A}

(Different letters in the same column shows significant differences. At day 0, retention of phytochemical properties was compared with respect to untreated SJ. Regarding day 7 – day 42, retention of phytochemical properties were compared with respect to the initial TPC, TAC, and RSA of SJ subjected to thermal (T), thermosonication (US), high pressure processing (HPP), and pulsed electric fields (PEF).)

Consequently, thermal pasteurization reduced TPC of SJ to 96 % while thermosonication did not change the TPC immediately after processing. Compared to thermal pasteurization, HPP and PEF treatments increased TPC to 104 and 105 %, respectively. At day 7^{th}, the initial TPC of HPP treated juice increased by 12 %. PEF treated samples also showed some increment in the TPC of SJ and achieved 106 % of retention at the first week of storage. Thermosonicated samples maintained the initial

TPC level at day 7 whereas thermally pasteurized caused 3 % of loss in the initial amount of total phenolic compounds. Then, total phenolic content of SJ samples irrespective of treatment underwent a substantial depletion until the end of storage period.

Nonetheless, the percent retention of TPC of T, US, HPP, and PEF treated samples at the end of storage (42^{nd} day) were recorded as 77, 76, 75, and 72 % with insignificant difference. The decrease in TPC in fruit juices during storage can be due to the insufficient inactivation of enzymes responsible for the degradation of phenolic compounds such as polyphenoloxidase, peroxidase and β-glucosidase (Barba et al., 2012).

7.3.3.2. Total Anthocyanin Content

Total anthocyanin content (TAC) of strawberry juice samples were also monitored during 42 days of storage (Figure 7.4). TAC of untreated SJ was 153.31 ± 2.57 mg pelargonidin-3-glucoside equivalent/L. While thermal pasteurization at 71.7 °C for 15 s and thermosonication at 55 °C did not significantly alter the content of total anthocyanins, HPP and PEF treatment increased TAC of SJ right after processing (day 0, $p<0.05$) (Table 7.2). During storage at 4 °C, TAC of strawberry juice samples showed a decreasing trend regardless of processing type (Figure 7.4).

Thermally pasteurized SJ samples contained lower amount of anthocyanins compared to US, HPP and PEF treated juices. These findings are in accordance with several other studies related to the stability of anthocyanins in fruit juices during storage. Since monomeric pelargonidin-3-glucoside (Pg-3-glu), cyanidin-3-glucoside (Cy-3-glu), pelargonidin-3-rutinoside (Pg-3-rut) are the major anthocyanins compounds in strawberry (Cao et al., 2011), they are widely analyzed by some researchers (Cao et al., 2012; Teleszko et al., 2016). Cao et al. (2012) reported total anthocyanins in HHP-treated cloudy and clear strawberry juices as 116.5 and 111.3 mg/L, respectively, before the storage. The retention of Cy-3-glu, Pg-3-glu, Pg-3-rut, and total anthocyanins in cloudy SJ was found to be 74.37%, 66.59%, 78.84% and 70.24% after 6 months of storage, and the values of the same compounds and total anthocyanins in clear juices were reported to be 90.1%, 94.13%, 91.11% and 92.98%, respectively. Significant losses in cloudy SJ were attributed to the higher concentrations of oxygen absorbed on

pulp particles promoting the degradation of anthocyanins (Cao et al., 2012). The findings obtained in this study related to the loss of anthocyanins in SJ during storage were found very similar to the findings of Cao et al. (2012). Loss of anthocyanins can be aroused from the oxidation as well as condensation of anthocyanins (Castaneda-Ovando, Pacheco-Hernandez, Paez-Hernandez, Rodriguez, & Galan-Vidal, 2009). It has been previously reported that condensation reactions of anthocyanins during storage occur due to the formation of complexes with other phenolics naturally occurred in juices such as strawberry and raspberry juices (Rein, Ollilainen, Vahermo, Yli-Kauhaluoma, & Heinonen, 2005).

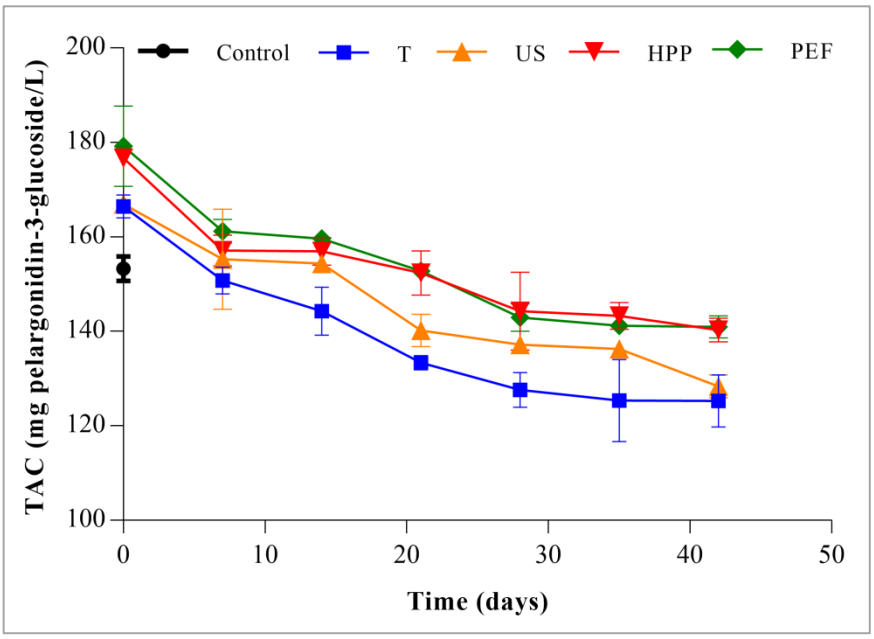


Figure 7.4. The effect of storage time on the total anthocyanin content (TAC) of strawberry juice subjected to different treatments
(T, US, HPP, PEF refer to thermal pasteurization, thermosonication, high pressure processing, pulsed electric fields, respectively)

In Table 7.3, retentions of TAC in SJ samples are given in terms of percentage in the same way as TPC. Retention percentages of TAC of treated SJ at day 0 were calculated considering TAC of untreated SJ as 100 %. On the other hand, the percent retention of TAC of treated SJ samples during storage starting from day 7th up to day 42nd was calculated considering the initial TAC of heat, US, HPP and PEF treated samples at day 0 as 100 %. Consequently, HPP and PEF treatments resulted in significant increment in the concentrations of total anthocyanins after processing compared to untreated juice ($p<0.05$). Even though anthocyanin concentration in

untreated SJ was increased by thermal pasteurization and thermosonication, the increment was not statistically significant. At the end of storage, TACs of juices subjected to thermal pasteurization, US, HPP, and PEF were 75, 77, 79, and 79 % with insignificant difference ($p>0.05$).

7.3.3.3. Antioxidant Activity

The quantitative changes in antioxidant activity right after the processing and during storage of SJ samples were measured using DPPH method and expressed in terms of radical scavenging activity (RSA) (%) (Figure 7.5 and Table 7.2).

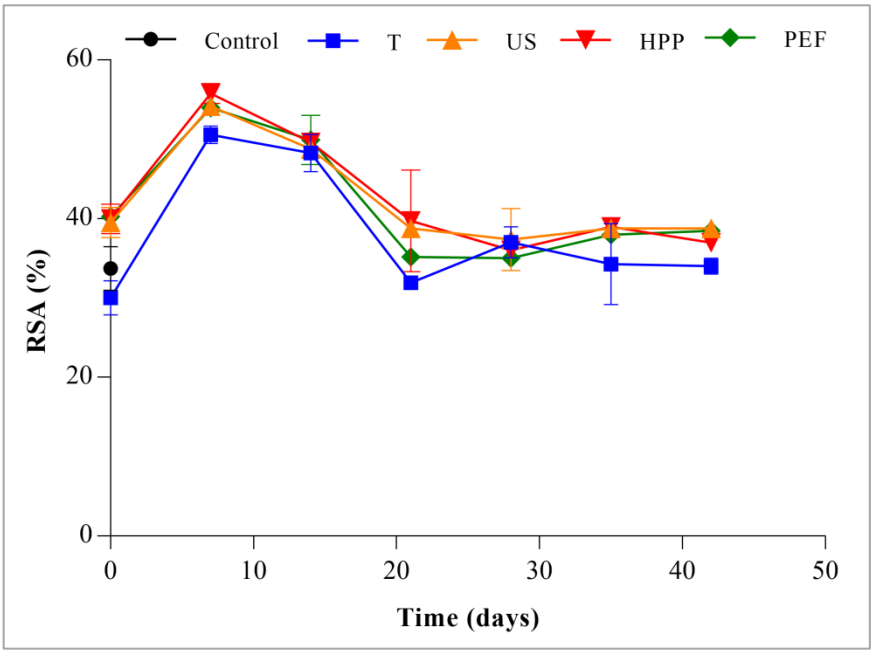


Figure 7.5. The effect of storage time on the radical scavenging activity (RSA) of strawberry juice subjected to different treatments
(T, US, HPP, PEF refer to thermal pasteurization, thermosonication, high pressure processing, pulsed electric fields, respectively)

In accordance with TPC results, HPP and PEF treated samples had higher antioxidant activity during storage period. The initial antioxidant activity of untreated SJ was found to be 33.72 ± 2.74 % (Table 7.2). The results were indicated that the values of antioxidant activity of SJ subjected to different treatments were not significantly different than the values of untreated samples at the end of storage period. However, HPP and PEF treatments retained the antioxidant activity of SJ samples

slightly better compared to thermal pasteurization. On the other hand, some significant fluctuations were observed for RSA values of SJ samples throughout storage at 4°C. For example, RSA of SJ samples subjected to heat, US, HPP, and PEF showed a significant increase at the 7^{th} day, and then a significant decrease until the 21^{st} day. Thereafter, SJ samples showed relatively consistent results with insignificant changes until the end of the storage.

Retention of antioxidant activities were evaluated in the same way as TPC and TAC as given in Table 7.3 in percentages. In conclusion, no significant difference was observed among the treatments during storage of SJ for a period of 42 days at refrigerated conditions. Nonthermal processing technologies resulted in better retention of the radical scavenging activities of SJ samples compared to untreated juice; however, storage time reduced the antioxidant activity in accordance with total phenolic and anthocyanin content. While SJ samples processed by nonthermal technologies contained higher antioxidant activities compared to thermally pasteurized juice at the beginning of the storage, the retention of RSA at the end of storage period were 114, 98, 93, and 96 % for thermal pasteurization, US, HPP, and PEF, respectively, and were not significantly different according to Tukey comparison test ($p<0.05$). Plaza et al (2006) processed orange juice with mild heat treatment (70 °C for 30 s), HPP (400 MPa, 40 °C for 1 min), PEF (35 kV/cm for 750 μs). Our findings were in good agreement with the results of their study. The authors stated that antioxidant activity of orange juice samples did not show any significant difference in terms of treatments after 40 days of storage at 4 °C. Therefore, HPP and PEF treatments were suggested as the effective technologies for the retention of antioxidant characteristics of orange juice during storage period (Plaza et al., 2006). Phenolic compounds and anthocyanins are natural antioxidants that can scavenge the free radicals in the media; thereby reduce the oxidative stress on human health. Thus, Martinez-Flores et al. (2015) pointed out that a preservation or enhancement of bioactive compounds in fruit juices by emerging technologies could increase the radical scavenging activity (Martinez-Flores et al., 2015) supporting the results of this study.

7.3.4. Principal Component Analysis

Principal component analysis (PCA) was utilized to visualize the data structure of the SJ samples during storage period. The structure of the whole data set obtained from storage study analyzed by principal component analysis (PCA) and depicted in Figure 7.6 and Figure 7.7. The data related to physicochemical (pH, titratable acidity, TSS) and phytochemical properties (TPC, TAC, RSA) of thermally pasteurized, thermosonicated, HPP and PEF-treated samples were used as PCA input. Control sample was only included at the beginning of storage (0^{th} day) since the microbial load increased afterwards. Score plot of PCA output shows the distribution and discrimination of treatments (Figure 7.6).

The first principal component (PC 1) was able to distinguish the samples stored up to 14 days by separating them from the rest of the stored samples. Samples of the day 0 (processing day) were located almost in the same region and close to each other. Samples of the 7^{th} day and 14^{th} day, on the other hand, fell close to each other except thermally pasteurized ones. The distribution of the treatments in terms of storage days was actually resulted from the physicochemical and phytochemical properties. Figure 7.7 is the loading plot indicating the distribution of physicochemical and phytochemical properties in space defined by the first and second PCA dimensions. Simultaneous evaluation of score and loading plot revealed that the contents of total phenolics, anthocyanins, and antioxidant activities of SJ samples were higher when the juice was stored up to 14^{th} day.

According to the second principal component (PC 2), nonthermal technologies located away from thermal pasteurization due to their higher content of phytochemical properties and titratable acidity as well as lower content of TSS and pH value compared to thermally pasteurized and untreated SJ. Thermosonicated and thermally processed samples showed similar properties while HPP and PEF treated juices were similar at the later days of storage (Figure 7.6 and Figure 7.7).

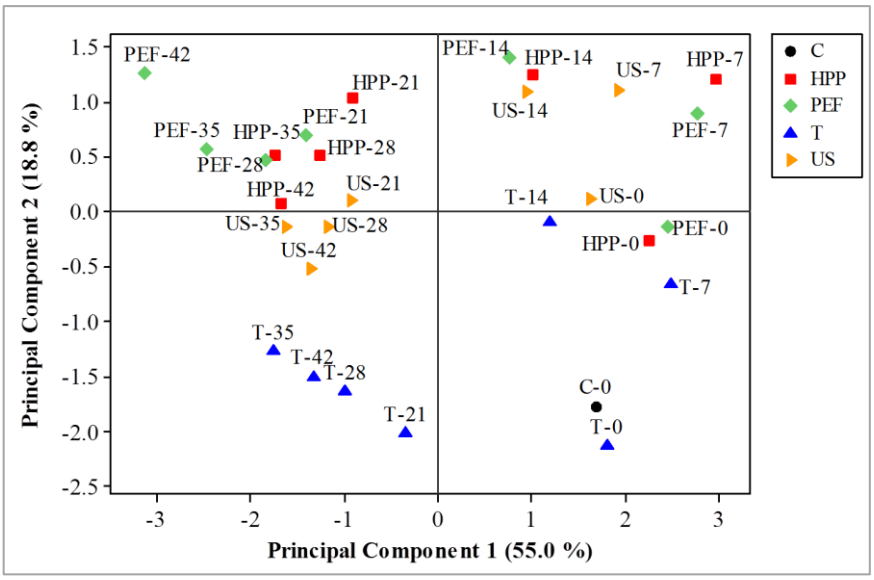


Figure 7.6. PCA output of storage data as score plot

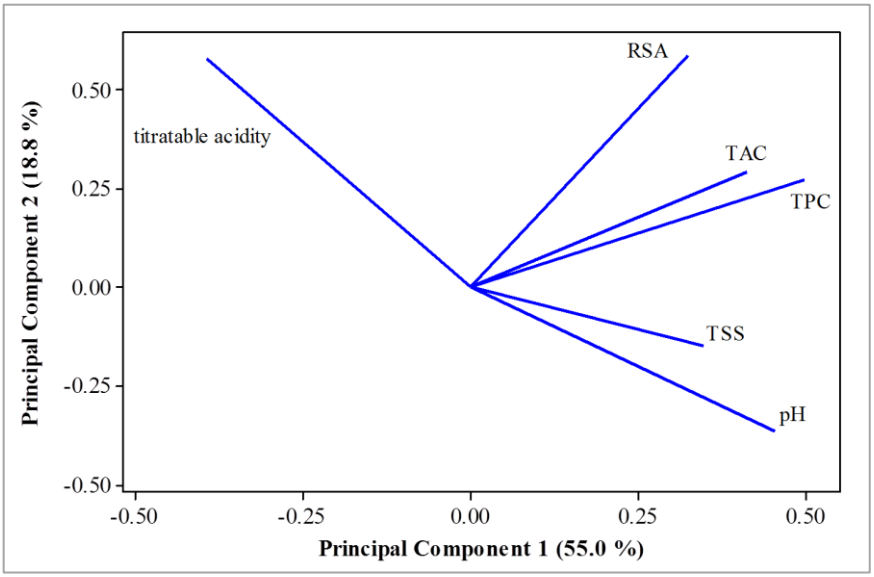


Figure 7.7. PCA output of storage data as loading plot

The loadings of PCA are given in Table 7.4. It is worth to mention that the values of phytochemical properties of SJ samples subjected to HPP and PEF treatments were high if evaluated with respect to the score and loading plots. All the factors had loading of greater than 0.3 for PC 1. However, TPC made the highest contribution (0.497). On the other hand, RSA and titratable acidity were the main loadings of PC 2 with a value of 0.590 and 0.580, respectively. Eigen analysis showed how much variance was able to be explained by each factor either in proportion or cumulative way.

The PC 1 was able to explain 55 % of the total variance while PC 2 accounted for 18.8 % of the total variance. Hence, PCA was able to cumulatively explain 73.8 % of total variance in this data set (Table 7.4). Multivariate data analysis tools such as PCA have been well applied by several researchers for the estimation of relationships between different innovative processing technologies and quality parameters of the treated products (Anaya-Esparza et al. 2017) including grapefruit juice (Aadil et al., 2015b), apple juice (Abid et al., 2014b), lemon melon juice blend (Kaya et al., 2015). Abid et al. (2014) were able to explain 84 % of the total variance by PC 1 and PC 2 for apple juice samples subjected to sonication and HPP. The authors stated that PCA was able to discriminate combined US-HPP treatment from the rest of treatments on the basis of ascorbic acid, phenolic compounds, and radical scavenging activity (Abid et al., 2014b). Kaya et al. (2015) were also able to distinguish untreated, UV-C or heat treated lemon-melon juice blends with respect to their physicochemical properties during storage of 30 days. Even though the level of explanation of total variance was lower (51.09%) compared to the current study, the authors were able to obtain a clear discrimination of treatments (Kaya et al., 2015).

Table 7.4. Loadings of PCA of storage study for each variable

Factor loadings	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC5**
TSS (°Brix)	0.346	-0.149	-0.696	0.596	0.129
pH	0.452	-0.367	-0.002	-0.494	0.645
Titratable acidity	-0.394	0.580	-0.129	0.089	0.660
TPC	0.497	0.273	0.212	0.095	-0.169
TAC	0.411	0.291	0.533	0.391	0.211
RSA	0.324	0.590	-0.411	-0.481	-0.242
Eigen analysis	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC5**
Eigen value	3.298	1.129	0.908	0.380	0.196
Proportion of variance	0.55	0.188	0.151	0.063	0.033
Cumulative variance	0.55	0.738	0.889	0.952	0.985
% Cumulative variance	55	73.8	88.9	95.2	98.5

7.3.5. Hierarchical Cluster Analysis

The scores of PCA model were used as an input for hierarchical cluster analysis (HCA) where Ward linkage and Euclidean distance was applied. Figure 7.8 shows the similarities and differences among juice samples subjected to different processing technologies and subsequent refrigerated storage. Untreated SJ at the processing day and treated SJ stored up to 14th day showed similar properties in terms of physicochemical and phytochemical properties by locating close to each other in dendrogram. In the same manner, SJ samples stored for 21, 28, 35, and 42 day showed similar properties by gathering together at the right side of the dendrogram. It was observed that thermal pasteurization and thermosonication located close to each other while HPP and PEF treatments settled closer in each cluster (Figure 7.8). Kaya et al. (2015) also used HCA for the classification of control, UV-C and heat treated lemon-melon juice blends considering their physicochemical properties such as pH, titratable acidity, TSS, turbidity, absorption coefficient, and color. HCA was able to reveal the similarities and differences among all blend samples during 30 days of storage (Kaya et al., 2015).

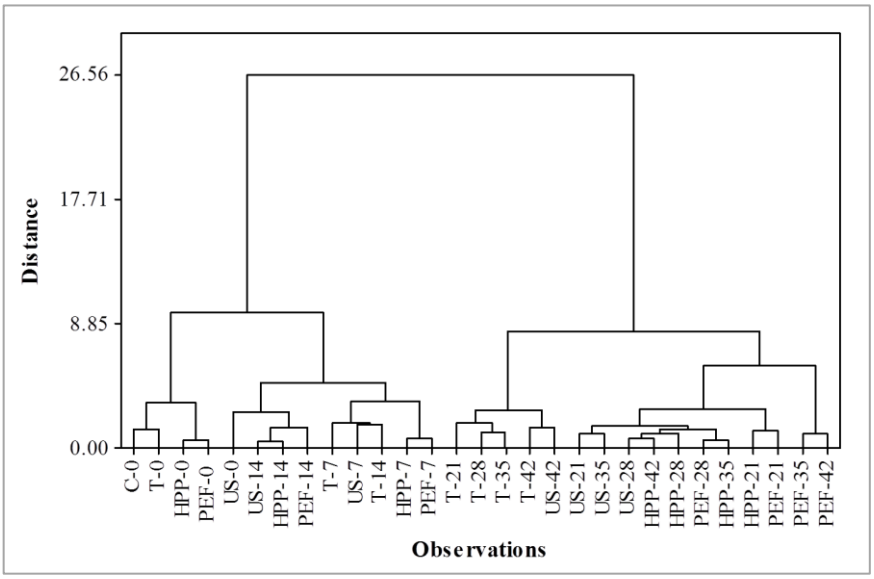


Figure 7.8. Dendrogram for the similarities and differences among juice samples during storage study
(Treatments at specific storage days are given on horizontal axis)

7.4. Conclusions

This chapter covered the microbial quality in terms of total mesophilic aerobic and yeast-mold counts as well as stability of physicochemical (pH, titratable acidity, TSS) and phytochemical properties (TPC, TAC, RSA) of strawberry juice subjected to thermal pasteurization (71.7 °C for 15 s), thermosonication at 55 °C (US), HPP at 300 MPa for 1 min, PEF (35 kV/cm, 27 μs, 155 Hz) and subsequent refrigerated storage at 4 °C for 42 days.

Microbial inactivation was ensured in this study as a prerequisite. While the TMAC and YM count of untreated strawberry juice exceeded the microbial criteria for the spoilage of fruit juices at 14^{th} day, thermal pasteurization, thermosonication, and HPP were able to maintain the microbial load at around 2 log CFU/mL during 42 days. In the case of PEF, a remarkable increase was observed at 35^{th} day. Thus, it can be concluded that the shelf life of untreated SJ was extended from 2 weeks to at least 6 weeks by thermal pasteurization, US, and HPP while PEF treatment prolonged the shelf life of SJ up to 5 weeks.

In addition ensuring the microbial safety in SJ subjected to different treatments, physicochemical characteristics of SJ were not affected from the treatments and found to be similar to each other. Even though some significant differences were observed in acidity and pH of the samples during storage; however, the numerical changes were in small range. No significant difference was observed in the total soluble solid content of the juice samples during storage ($p>0.05$).

Phytochemical properties in terms of TPC, TAC, and antioxidant activity (RSA) were significantly decreased by thermal pasteurization compared to HPP and PEF right after processing. However, irrespective of treatment types, a remarkable decrease was observed for the phytochemical attributes of SJ after the 7th day of refrigerated storage. This can be resulted from the residual enzymes that can have a role on degradation of antioxidant compounds. Thus, a future study can be suggested for investigation of the enzyme inactivation in SJ under equivalent conditions.

Multivariate data analysis tools such as PCA and HCA were able to distinguish the SJ samples considering treatments types and storage days. The classification was based on the all physicochemical and phytochemical data obtained in the storage study of SJ.

In conclusion, mild heat treatment at moderate temperature, thermosonication, HPP, and PEF processes can be suggested as alternative methods to thermal pasteurization for the extension of microbial shelf life of strawberry juice. Additionally, preservation or enhancement of bioactive compounds in fruit juices can be achieved by using emerging nonthermal technologies.

CHAPTER 8

PASTEURIZATION OF STRAWBERY JUICE BY UV-C IRRADIATION AND A COMBINED UVC-MILD HEAT TREATMENT

8.1. Introduction

Thermal pasteurization has been conventionally applied in order to extend the shelf life of fruit juices and ensure the product safety. For this purpose, low temperature long time (LTLT) or high temperature short time pasteurization methods has been used extensively in the field of food processing (Koutchma, Popovic, Ros-Polski, & Popielarz, 2016). Nonthermal processing technologies have been emerged as alternative methods to reduce any possible heat damage on fresh-like properties and nutrient composition of the product (Sanchez-Moreno, De Ancos, Plaza, Elez-Martinez, & Cano, 2009). UV-C irradiation has been considered as one of the nonthermal technologies having germicidal effect against different microorganisms such as bacteria, viruses, protozoa, yeast, molds, and algae (Gayán, Condón, & Álvarez, 2014; Koutchma, 2009b). The U.S. Food and Drug Administration (FDA) introduced regulation (21 Code of Federal Regulations 120; FDA, 2001) mandating that all 100% fruit/vegetable juices sold wholesale be produced under a Hazard Analysis and Critical Control Point (HACCP) plan. This regulation requires that juice processors obtain at least 5 log reduction in the most pertinent microorganism in the treatment medium (FDA, 2001). Afterwards, in 2004, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of USDA redefined the pasteurization term as “any process, treatment, or combination thereof, which is applied to food to reduce the most microorganism(s) of public health significance” (NACMCF, 2004).

The objective of this chapter was to determine the processing conditions for pasteurization of strawberry juice (SJ) by UV-C irradiation alone and a combined UVC-mild heat treatment. For this purpose, freshly squeezed SJ was subjected to thermal pasteurization in order to eliminate the background flora in the juice. Following the

pre-pasteurization step, strawberry juice was inoculated with a target microorganism, i.e., *E. coli* K-12 as a surrogate of *E. coli* O157:H7, and exposed to UV-C irradiation, and combined UVC- mild heat treatment. In accordance with the FDA's 5-log reduction performance standard (FDA, 2001), the conditions resulted in at least 5-log reduction of *E.coli* K-12 were chosen as the final processing conditions. Untreated and thermally pasteurized juice samples were used as negative and positive controls, respectively, for comparison against juice samples treated with UVC irradiation and combined UVC-mild heating.

8.2. Materials and Methods

8.2.1. Experimental Procedure

Figure 8.1 shows the flow diagram of the studies conducted in this chapter. SJ juice samples were prepared similarly according to the method described in Chapter 4.

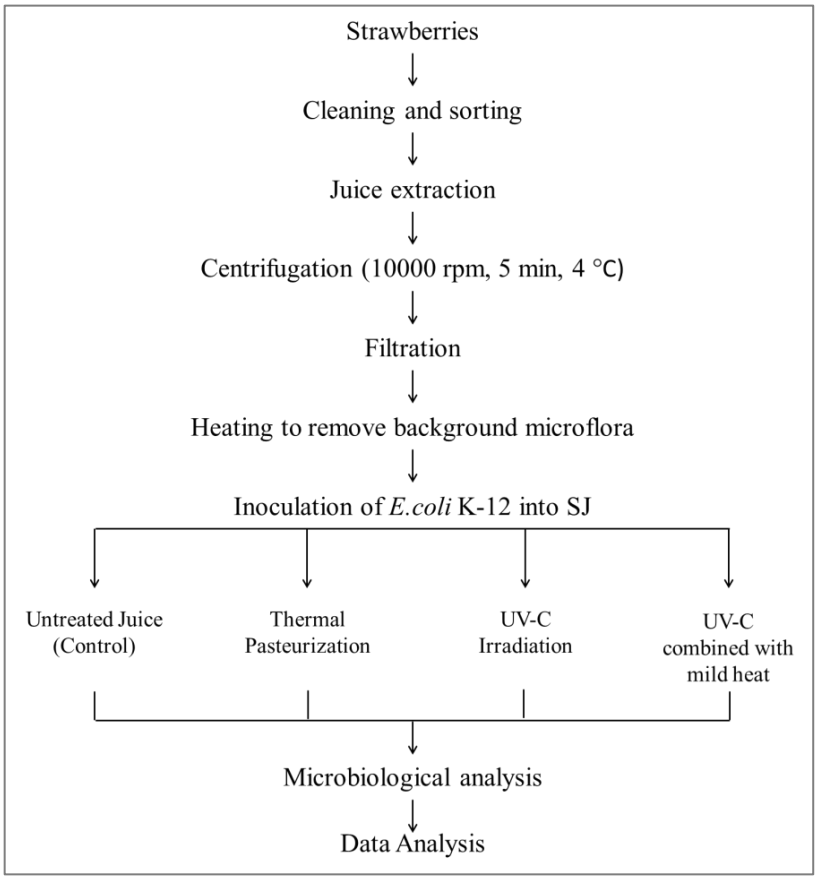


Figure 8.1. Flow diagram of Chapter 8

Briefly, strawberries were firstly cleaned and sorted. The juice was extracted from the fruits by a fruit juice extractor and centrifuged at 10000 rpm and 4 °C for 5 min. Then, it was filtered through a sterile gauze strip to eliminate the suspended particles. The filtered strawberry juice was then preheated in order to eliminate the background microflora and cooled down to ambient temperature by placing the pre-heated juice in ice water. Thereafter, the strawberry juice was inoculated with *E.coli* K-12 (ATCC 25253) and exposed to further treatments such as thermal pasteurization, UV-C irradiation, combined UV-C-mild heat treatment (UV-C-MH). The effects of treatments on the inactivation of *E. coli* K12 in SJ were then evaluated and compared with untreated juice samples.

8.2.2. Pasteurization of Freshly Squeezed Strawberry Juice (Pre-Pasteurization)

Before inoculation of SJ with target *E. coli* K-12 (ATCC 25253) cells, the juice was pre-pasteurized at 70 °C in order to eliminate the background microflora. For this purpose, SJ was heated up in boiling water as illustrated in Figure 8.2. 800 mL of water in 2 L of beaker was placed on a hot plate adjusted to 300 °C and 250 rpm. When the water reached the boiling temperature, 400 mL of juice (20 °C) in the Schott bottle was placed into the boiling water and heated to 70 °C in 5 min. The juice was kept at this temperature for 15 s; and subsequently cooled down to approximately 20 °C. Then the pre-pasteurized juice was used for inoculation and inactivation experiments.

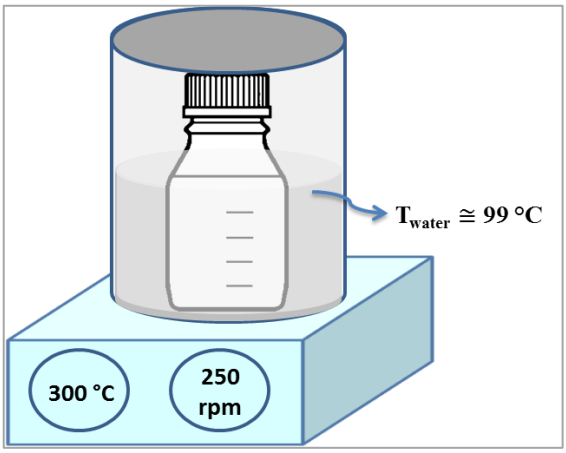


Figure 8.2. Schematic view of the pre-pasteurization of the juice prior to sample inoculation

8.2.3. Bacterial Strain, Sample Inoculation and Bacterial Enumeration

Koutchma, Keller, Chirtel & Parisi (2004) found that UV sensitivities of *E. coli* O157:H7 and a surrogate *E.coli* K12 (ATCC 25253) were not significantly different from each other. Thus, *E. coli* K-12 (ATCC 25253), a surrogate of *E. coli* O157:H7 was used as a target microorganism for inoculation of juices. The *E. coli* K12 (ATCC 25253) strain was cultured from −80 °C lyophilized vials, enriched in a test tube containing nutrient broth (NB, Merck, Darmstadt, Germany) and incubated overnight (18–24 h) at 37 °C. The *E. coli* K12 (ATCC 25253) culture was first adapted to pH 4.0 by growing in a solution containing tartaric acid following the procedure described by Pala & Toklucu (2013a). For this purpose, *E.coli* cells was incubated at 37 °C during 18 h by using Tryptone soy broth (TSB, Merck, Germany) at pH 7.0. Acid adaptation procedure was then carried out by lowering the pH gradually. 1 mL of the initial culture was transferred to TSB adjusted to pH 6.5 and incubated for 18 h at 37 °C. Then 1 mL of pH 6.5 adapted culture was transferred to TSB adjusted to pH 6.0. In the same manner, the adapted culture was transferred to a new media adjusted to a lower pH considering the order of pH 5.5, pH 5.0, pH 4.5, and finally pH 4.0 (Pala & Toklucu, 2013a). The last culture adapted to pH 4.0 was used throughout the inactivation studies. Stock cultures were prepared by transferring acid-adapted cells onto TSA slants and stored at 4°C until used. A loopful of acid-adapted culture from the TSA slant was first inoculated into 10 ml of TSB (TSB, Merck, Darmstadt, Germany) for enrichment, and then 1 mL from this culture medium was inoculated into SJ.

Inoculated SJ was then subjected to thermal processing, UVC irradiation and combined UVC-mild heating. To enumerate the viable microorganisms in the treated and untreated samples, appropriate dilutions were made with 0.1 % peptone water and surface plated in duplicate on tryptic soy agar (TSA, Merck, Darmstadt, Germany) plates. All of the plates were incubated at 37 ºC for 18 h and then counted. The untreated SJ sample was used as a negative control, while a heat treated sample was used as a positive control in this study. The treated, positive and negative control samples were checked by surface plating on tryptic soy agar (TSA, Merck, Darmstadt, Germany) containing 0.1% dihydrostreptomycin (Koutchma, Keller, Chirtel, & Parisi, 2004) i.e., an antibiotic agent to which *E. coli* K-12 is resistant. TSA plates were

incubated at 37 °C for 18 h. Results were expressed as colony-forming units (CFU) per milliliter.

8.2.4. Determination of Thermal Kinetic Parameters and Pasteurization Conditions

Thermal kinetic parameters were utilized to determine thermal processing conditions for ensuring the microbial safety and preservation of quality of the product (Erkmen & Barazi, 2012). Chick (1908) observed the exponential decay in the microbial survivor curves and mathematically described the inactivation of microorganism population by a first-order reaction (Chick, 1908). The following equation was used to describe the first order inactivation kinetics (Equation 8.1) (Chick, 1908; Watson, 1908).

$$\log_{10}(\frac{N}{N_0}) = -kt \tag{8.1}$$

where N_0 is the initial microbial load, N is the concentration of microorganism at a specific time (CFU/mL), k is the inactivation rate constant (1/time). Equation 8.1 can be written in the following form as well (Stumbo, 1973).

$$\log N = \log N_0 - \frac{t}{D} \tag{8.2}$$

where D = 2.303/k.

D value (decimal reduction time) has been defined as the time required at a certain temperature to inactivate 90% of the organisms being studied (Peleg, 2006). Following equation was used for the calculation of D-values of target microorganism (Stumbo, 1973).

$$D = \frac{t_2 - t_1}{\log N_1 - \log N_2} \tag{8.3}$$

where t is the holding time, and N is the viable cell number.

D value varies with temperature. A linear correlation which is called as thermal death time curve was obtained by plotting the log-D values versus temperature. The use of thermal death time curve reveals the necessary temperature increase to obtain 1-log reduction in D value. This is called as z-value as represented in the Equation 8.4.

$$z = \frac{T_2 - T_1}{\log D_{T_1} - \log D_{T_2}} \tag{8.4}$$

where T_1 and T_2 are two different temperatures, D_T is the D value measured at a specific temperature. The linear regression equation of log D-value versus temperature reveals z-value by calculating -1/slope.

Initially, thermal inactivation kinetic parameters (D and z values) of the target microorganism in SJ were determined by constructing a thermal death time curve. For this purpose, *E. coli* K-12 (ATCC 25253) cells, previously adapted to pH 4, were used in this study. Initially, strawberry juice samples (3.9 mL) were placed into glass test tubes. The tubes were then heated to certain temperatures (70-85 °C) in a water bath adjusted to corresponding temperatures. Once the temperature of juice samples reached to the desired degree, 0.1 mL of *E.coli* was added; and inoculated juice samples were subsequently kept at the desired temperature up to 180 s.

After the heating process, the survived *E. coli* K-12 cells were plated on Tryptic Soy Agar (TSA) in duplicate by spread plate method considering appropriate dilutions with phosphate buffered peptone solution. Residual colonies were enumerated after incubation at 37 °C for 18 h. Results were expressed as log CFU/mL.

The initial concentration of *E.coli* in each test tube was approximately 6-7 log CFU/mL. The reduction in the initial number (N_0) of population was monitored during 5-180 s heating at constant temperatures. Survival curve of viable cells was constructed by plotting log (N) versus corresponding time. D values for each temperature (70-75-85 °C), were then calculated by Equation 7.3. Log D-values were plotted against temperature and 1-log reduction in D value was calculated by -1/slope of linear regression line.

The z value indicates the degree of temperature required for the one log reduction of D value (Erkmen & Barazi, 2012). The pasteurization time ensuring 5-log reduction of *E.coli* in SJ at 72 °C was calculated based on D and z values. All the

measurements in this study conducted to determine the thermal kinetic parameters were performed in duplicate.

8.2.5. Thermal Pasteurization of Strawberry Juice

Thermal pasteurization was conducted in duplicate based on the findings of thermal kinetic parameters study. 72 °C and 101 s were chosen as pasteurization conditions. Strawberry juice was thermally pasteurized by following a continuous flow as highlighted in Figure 8.3. The juice was placed in a sample tank and water at 70 °C circulated around it. Strawberry juice was then pumped (13 rpm) to helical tube placed in water bath. The flow rate and the residence time inside the tube were 0.72 mL/s and 101 s, respectively. The pasteurized samples were collected and used for further analyses.

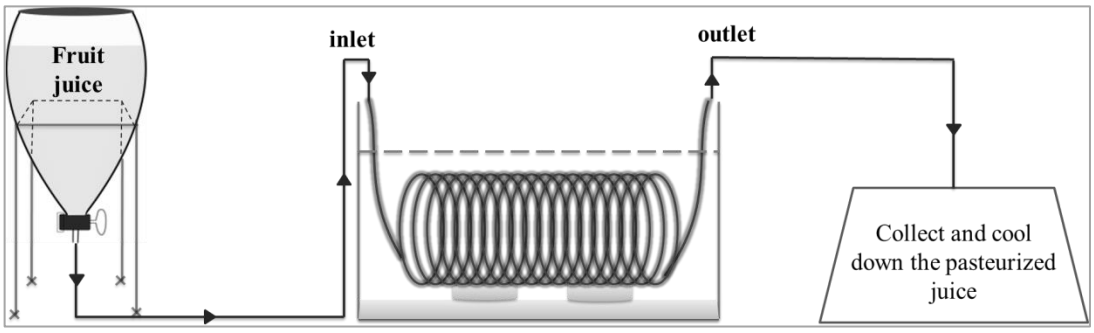


Figure 8.3. Scheme of thermal pasteurization

8.2.6. UV-C Treatment of Strawberry Juice

The UV system was designed and manufactured at Department of Food Engineering, Izmir Institute of Technology (Figure 8.4 and 8.5). UV system was made of a glass storage tank equipped with a cooling jacket, a pump (Watson Marlow Inc., England) used to regulate the volumetric flow rate, and an annular quartz glass tube surrounded by a cylindrical aluminum reflector (Afe Olgunlar Inc., Turkey) and seven UV-C lamps (254 nm, 15 W UV-C output, UVP XX-15, UVP Inc., CA, USA). One of the UV lamps was located in the middle of the quartz tube, whereas the other UV lamps

were assembled around the quartz tube (Kaya, Yildiz, & Unluturk, 2015; Unluturk & Atilgan, 2014). The gap size for annular flow was approximately 5 mm.

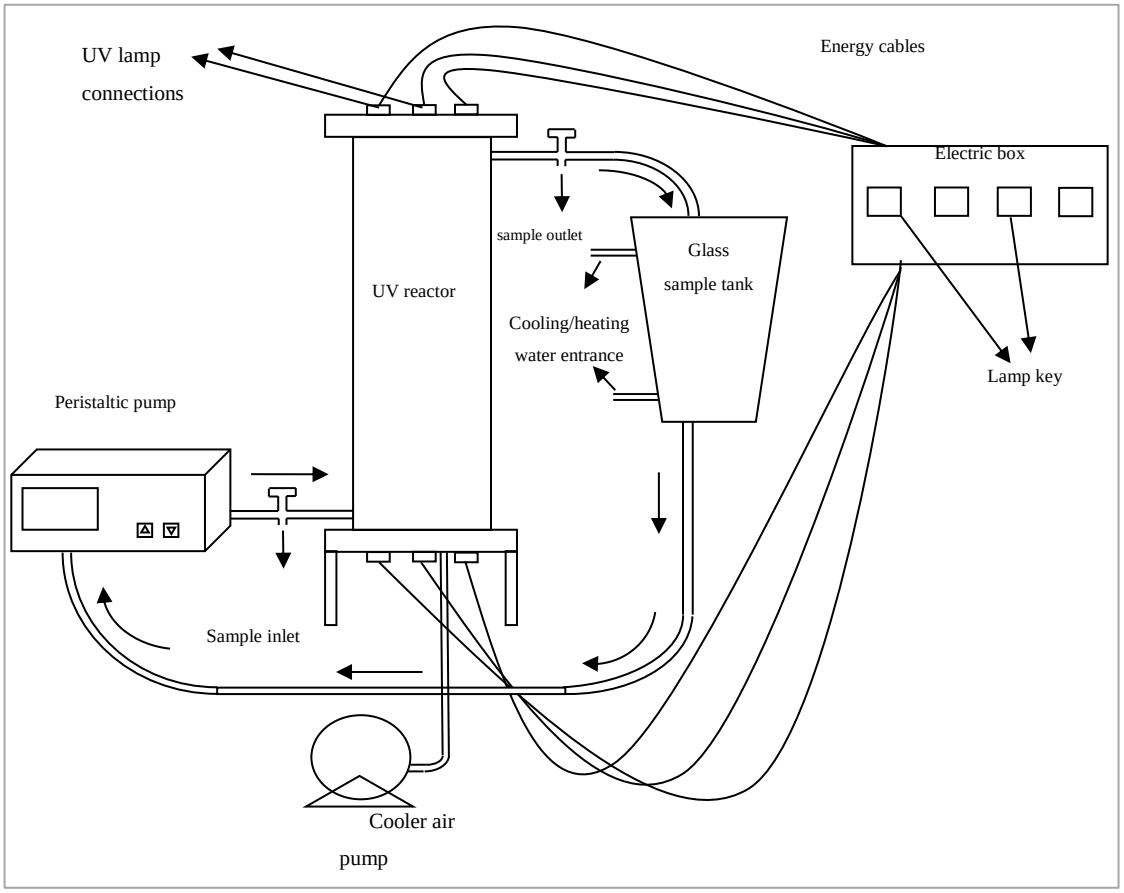


Figure 8.4. A general schematic drawing of continuous flow annular UV system (Source: Unluturk & Atilgan, 2014)

Four hundred mL of juice samples inoculated with approximately 6.10 ± 0.29 log CFU/mL of *E. coli* K-12 passed through the UV-C system circulating cold water at 4 ºC around the glass sample tank. The water around the glass 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 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). UV treatments were carried out at two flow rates (3.80 mL/s, 7.55 mL/s). Time necessary to complete one cycle of 400 ml SJ in the UV-C system was recorded manually at each flow rate by using a chronometer. Thus, the total processing time was calculated by summing up t_1 (the time required for the transportation of sample from sample tank to the entrance of UV-C reactor), t_2 (the time that the SJ samples are subjected to UV-C light, also known

as UV exposure time), t_3 (the time required to remove all the juice away from the system) (Figure 8.5).

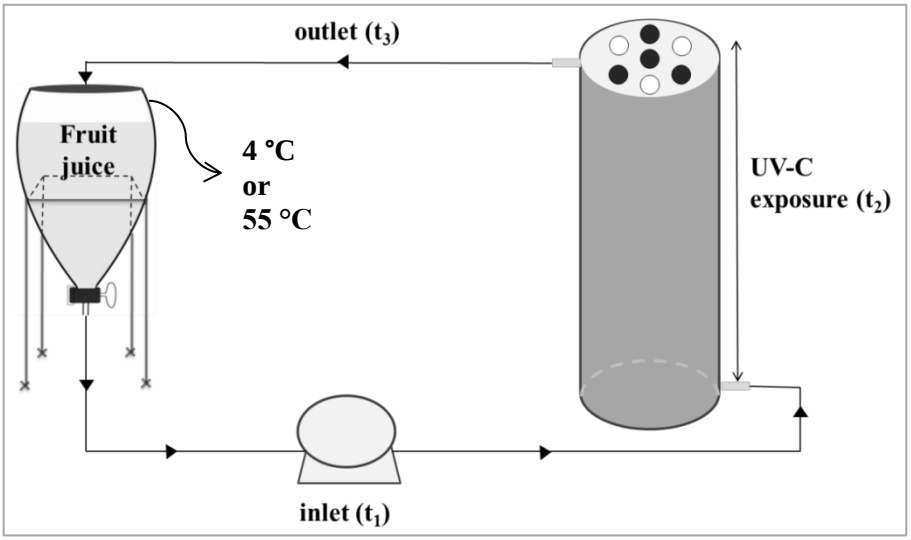


Figure 8.5. Continuous flow UV system with flow direction

Different lamp configurations and flow rates can deliver different amount of UV light intensity to the fluid circulating in the UV system. Thus, the effect of two different lamp configurations (4 UV and 7 UV lamps turned on) and two different flow rates (3.80 mL/s and 7.55 mL/s) on the inactivation of *E. coli* K12 in SJ were investigated. For this purpose four different designs including I (4 UV lamps on - 3.80 mL/s flow rate), II (4 UV lamps on - 7.55 mL/s flow rate), III (7 UV lamps on -3.80 mL/s flow rate) and IV (4 UV lamps on - 3.80 mL/s flow rate - mild heating) (Figure 8.6). Firstly, inoculated juice samples were circulated through the reactor using design I and II. With respect to the results of design I and II, third design (III) was constructed in order to study the inactivation level when all the lamps were switched on at a low flow rate (3.80 mL/s). The last configuration (IV) was designed to investigate the synergistic effect of UV-C and mild heating on the inactivation of *E. coli* K12 in SJ. Fifty five degree Celsius has been previously reported as the optimum temperature to benefit the synergistic effects of UV and mild heat (Gayan, Serrano, Monfort, Alvarez, & Condon, 2013). Four hundred mL of juice was firstly pre-heated to 52 °C by placing the juice bottle into boiling water as shown in Figure 8.2. Then the pre-heated juice was transferred to the sample tank of the UV system. In this case, hot water adjusted to 55 °C was circulated through the double walled sample container.

The juice was circulated eight times through the UV system. All experiments were duplicated and sampling was performed after each cycle for each design. The samples drawn after each cycle was cooled down in an ice-water bath and plated on TSA for enumeration of *E.coli* K-12 survived after UV processing. The survival curve of microorganisms was plotted by using logarithmic reductions i.e. log (N/N_0) 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).

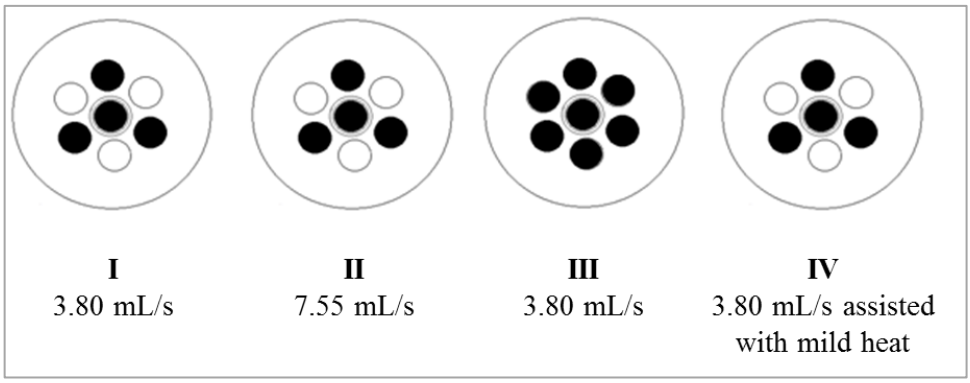


Figure 8.6. UV-C Lamp configurations (black: lamps on)

8.2.7. Determination of UV-C Dose

Actinometry is a method for the determination of the photon flux for a specific system. Chemical actinometer can be defined as the reference substance that has role in a photochemical reaction. Most common chemical actinometers are ferrioxalate or iodide-iodate solutions (Atılgan, 2013; Rahn, 1997).

The applied UV-C incident intensities were estimated by using a potassium iodide/iodate actinometer (Rahn, 1997). The actinometer buffer was pumped through the UV-C system at each flow rate and lamp configuration. The chemical actinometer was 0.6 M potassium iodide / 0.1 M of potassium iodate solution in 0.01 M borate buffer (pH 9.25) (Merck, Germany). Triiodite was formed due to the irradiation of potassium iodide/iodate solution. The concentration of triiodide was quantified by measuring its absorbance at 352 nm for each configuration.

Before UV-C irradiation, the initial optical density of the original KI solution was measured at 300 nm. Then, the initial amount of iodide concentration was calculated by Equation 8.5:

$$[c_{iodide}](M) = \left.\frac{OD}{\varepsilon * l}\right|_{@300\ nm} \tag{8.5}$$

where ε was the extinction coefficient and equal to 1.061 [1/(M*cm)] and *l* referred to the path length which is equal for 1 cm.

After photocatalytic reaction, Equation 8.6 was used for the determination of triiodide concentration (Rahn, 1997):

$$[c_{triiodide}](M) = \left.\frac{OD}{26400\frac{1}{M\ cm} * l}\right|_{@352\ nm} \tag{8.6}$$

where 26400 [1/(M*cm)] is molar extinction coefficient of triiodide formed in a cell having 1 cm path length. Total number of moles of triiodide formed was found by Equation 8.7:

$$n_{triiodide} = c_{triiodide} * V_{sample} \tag{8.7}$$

Quantum yield is defined as the ratio of number of absorbed molecules to the number of photons absorbed per unit time. It identifies the rate of the photochemical reaction. Quantum yield (Φ) was determined according to Rhan (1997) as given in Equation 8.8:

$$\Phi = 0.75[1 + 0.02(T - 20.7)][1 + 0.23(c_{iodide} - 0.577)] \tag{8.8}$$

The number of einsteins of photons absorbed by the sample was found by dividing the moles of product with the quantum yield (Equation 8.9):

$$E\left(\frac{einsteins}{L}\right) = \frac{\Delta OD_{352\ nm} * V_{sample}}{26400 * \Phi} \tag{8.9}$$

Assuming that all photons absorbed at 254 nm of wavelength, the total energy absorbed by the sample (incident joules) can be determined considering Equation 8.10:

$$Incident\,joules = \frac{4.72*10^5\,J}{Einsteinphotons} * E\left(\frac{Einstein}{L}\right) \tag{8.10}$$

The rate of UV exposure was then calculated by dividing the incident joules by volume of annular gap:

$$UV\ Incident\ \ Intensity\ \ (J/mL) = \frac{incident\ joules}{Volume} \tag{8.11}$$

Thus, the number of einsteins of photons absorbed by the irradiated sample were calculated from the moles of generated triiodide (Rhan, 1997). The photon flux (254 nm) received per volume unit and the corresponding effective dose was estimated.

8.2.8. Modeling of UV-C Inactivation Kinetics of *E. coli* K-12 in Strawberry Juice

In order to study the inactivation kinetics of a UV dose response curve, linear and non-linear inactivation kinetic models can be used. Several inactivation behaviors can be observed for microorganisms exposed to thermal or nonthermal food processing applications. Some of the inactivation curves are illustrated in Figure 8.7 (Geeraerd, Valdramidis, & Van Impe, 2005). Depending on the physiological condition and population of organism as well as the destructive potential of lethal agent, survivor curves with various shapes such as concave, convex, sigmoid and linear may be obtained (Casolari, 1988).

Log-Linear model is widely accepted and used to describe the microbial inactivation resulted from application of both thermal and non-thermal processes. This model assumes that the inactivation of microorganisms obeys the first order kinetic model (Peleg, 2006). The model is given in Equation 8.1 (Chick, 1908; Watson, 1908).

$$\log_{10}\left(\frac{N}{N_0}\right) = -kt$$

where N_0 is the initial microbial load, N is the concentration of microorganism at a specific time (CFU/mL), k is the inactivation rate constant (1/time).

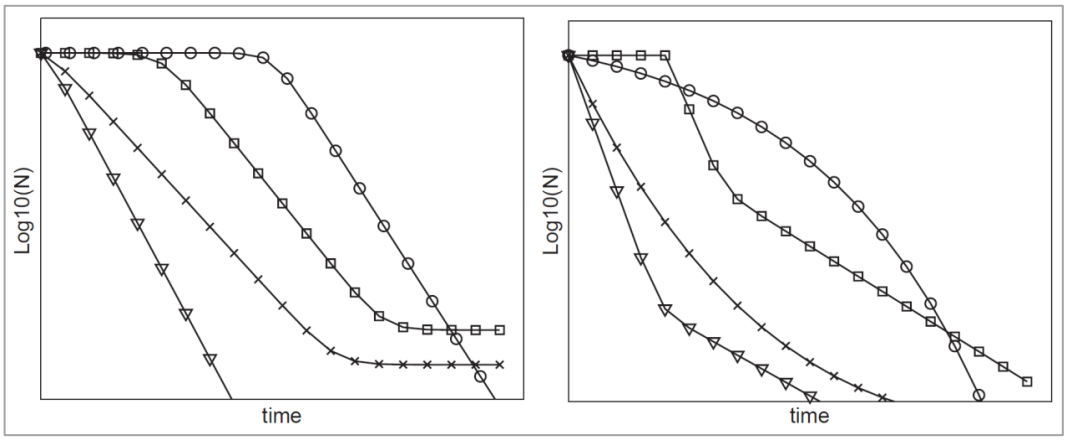


Figure 8.7. Representation of different survival curves of microorganisms (Source: Geeraerd et al., 2005)

Nonlinear inactivation curves are mainly composed of three different regions when there is a resistivity to inactivation of target microorganism: (i) a delayed response at the beginning of lethal effect is called as “shoulder”, (ii) a log linear inactivation region, (iii) residual survival at the end of process refers to “tailing” (Marugan, van Grieken, Sordo, & Cruz, 2008).

A single cell can be affected by multiple UV contacts or single UV light can have influence on a group of cells. In such cases, inactivation efficiency of UV light can decrease. This phenomenon is called as multiple hit. Therefore, less resistant cells are eliminated first, then more resistant cells are left to form a tail in survival curve (Bialka, Demirci, & Puri, 2008; Buzrul & Alpas, 2007; Gomez, Garcia, Alvarez, Condon, & Raso, 2005; van Boekel, 2002). Weibull model has been widely used to predict the nonthermal inactivation behavior of microorganisms. It describes sigmoid curves and exhibits concavity or convexity behaviors through downwards or upwards as a function of inactivation time or UV dose (Albert & Mafart, 2005; Stone, Chapman, & Lovell, 2009). It is described by the following equation:

$$\frac{N}{N_0} = 10^{-(\frac{t}{\delta})^p} \tag{8.12}$$

where δ is a scale parameter p refers to the shape parameter or concavity index.

The reliable time (t_R) indicates the time required for 90 % reduction of the number of microbial cells (van Boekel, 2002).

$$t_R = \delta.(2.303)^{1/p} \quad (8.13)$$

First-order kinetics is actually a special case of the model when p equals to 1. A concave upward survival curve is obtained when p<1. On the other hand, a downward concavity has been seen in the case of p>1 (Peleg, 2006). Upward concavity of the inactivation curve is an indicator of stress adaptation of the target microorganism survived after UV exposure while the downward concavity represents accumulated damaging effect of UV in the cells (van Boekel, 2002)

In the current study, the processing conditions for UV-C irradiation of strawberry juice were determined based on the inactivation study of *E. coli* K-12. Inactivation data were evaluated by GInaFiT (Geeraerd and Van Impe Inactivation Model Fitting Tool). Two approaches, i.e. log-linear and Weibull models, were considered for the mathematical modelling of inactivation data in order to estimate the inactivation behavior of the target microorganism.

8.2.9. Data Analysis

All treatments were conducted in duplicate. One-way analysis of variance (ANOVA) was used in order to determine how significantly the independent variables (treatments) affect the changes observed in the dependent variable (logarithmic reduction of *E.coli* K-12 in SJ). The means were compared by Tukey's comparison test. Data analyses were carried out by Minitab 16 (Minitab Inc., State College, PA, USA) and a data sheet (Microsoft Excel, 2010). Results were expressed as means ± standard deviation. Regression analysis was performed for the thermal kinetic parameters (D and z) and UV inactivation data by using Excel data sheet. Inactivation data were modelled by using GinaFit modelling Excel add-in application (Geeraerd et al., 2005). Shape and scale parameters, the root mean squared error (RMSE) and coefficient of determination (R^2) values obtained by GinaFit were compared in order to determine the suitability of fitted functions for Weibull distribution and Log-linear model. Equation 8.14 shows the formula for the calculation of RMSE.

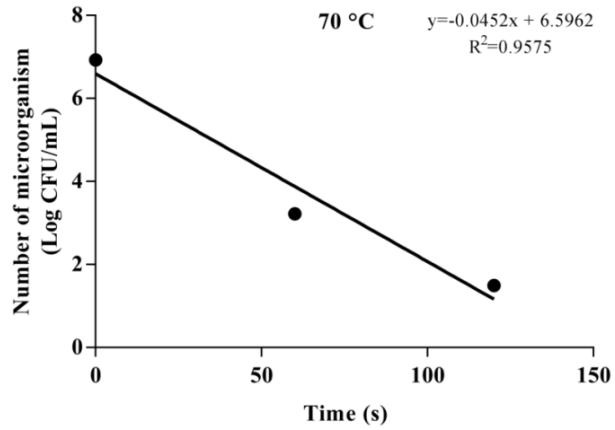
$$RMSE = \sqrt{\frac{1}{n}\sum\left[\left(\log\frac{N}{N_0}\right)_{cal} - \left(\log\frac{N}{N_0}\right)_{\exp}\right]^2} \quad (8.14)$$

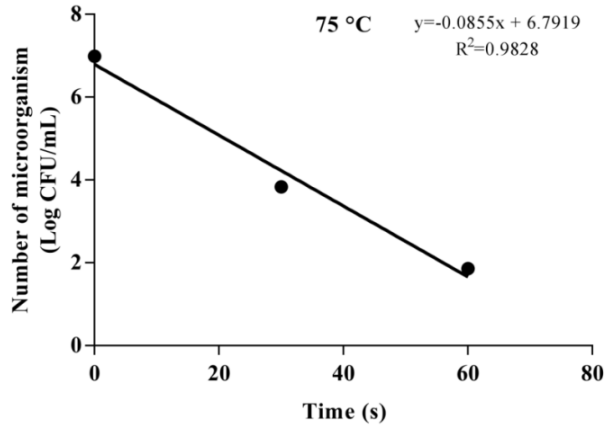
8.3. Results and Discussion

8.3.1. Thermal Pasteurization

The reduction in the population of acid adapted *E.coli* K-12 due to thermal pasteurization was evaluated by plotting the logarithm of survivor cells (log CFU/mL) versus time (s). Figure 8.8 represents the survivals at each temperature. Log D-values were calculated from this plot considering the Equation 8.3. Afterwards, log D-values were plotted against corresponding temperature which is called as thermal death curve (Figure 8.9). D value for *E.coli* K-12 at 72 °C ($D_{72°C}$) was found to be 20.21 s. Therefore, FDA's requirement of 5 log reduction of *E. coli* was achieved by thermal pasteurization after 101.07 s.

Heat resistance of acid adapted *E. coli* O157:H7 at 60 °C was reported to be 1.5, 1.7, and 1.2 min (D-value) for apple, orange, and white grape juice, respectively (Mazzotta, 2001). In another study, D-value of *E. coli* O157:H7 was given as 1.0 min at 58 °C in apple cider (Splittstoesser, McLellan, & Churey, 1996). However, thermal inactivation of *E. coli* K-12 was conducted at different temperatures varying from 70 to 85 °C in the current study. Thus, the cells were actually subjected to more lethal temperatures. Therefore, the varying findings related to the heat resistance of *E. coli* could be due to the strain of *E. coli*, growth phase of microorganism (Mazzotta, 2001), pH of the medium (Splittstoesser et al., 1996), use of different techniques and materials such as glass tubes (Chung, Wang, & Tang, 2007). Besides, acid adaptation of target microorganism cross-protects the cell by increasing the D-values. Therefore, determination of thermal pasteurization conditions considering heat resistance of acid adapted cells would provide more safety (Mazzotta, 2001).





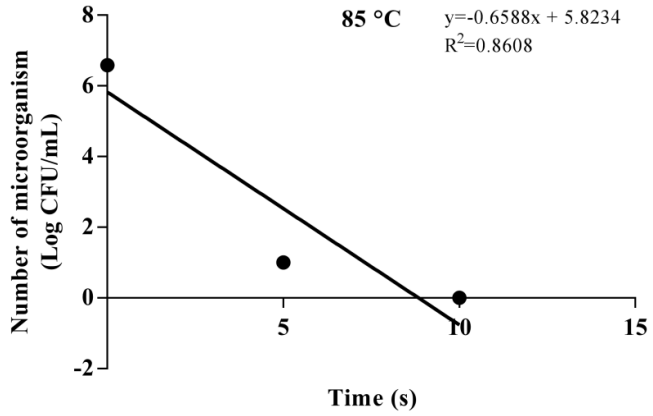


Figure 8.8. Reduction in the number of *E. coli* K-12 in SJ at different temperatures

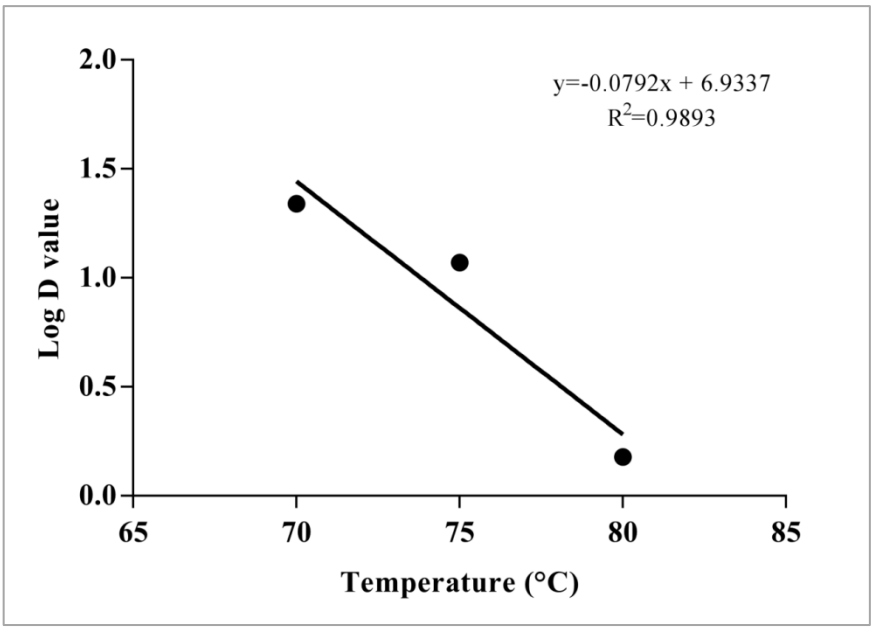


Figure 8.9. Thermal death curve for *E.coli* K-12 inoculated into SJ

8.3.2. UV-C Processing

Reduction in the number of inoculated *E.coli* K-12 cells by design I, II, III, IV are shown in Figure 8.10. Initial load of *E. coli* was approximately 6 log CFU/mL. Inactivation levels of *E.coli* K-12 by 6 cycles using design I and II were found as 1.04 ± 0.30 log and 0.82 ± 0.00 log, respectively. Although relatively higher inactivation rate was obtained with lower flow rate (3.80 mL/sec), none of the designs were able to satisfy the FDA's requirement of 5 log reduction of target microorganism. Therefore, in the design III, all the lamps were used at low flow rate of 3.80 mL/s. However, it was found that even the maximum numbers of UV lamps were used, FDA's requirement was not satisfied. Only 0.99 ± 0.23 log reduction was achieved by design III (Figure 8.10). The logarithmic reductions in the number of *E.coli* K-12 cells after each cycle for each design are given in Table 8.1. For each flow rate, the processing time for one pass was recorded as 113 s at 3.80 mL/s and 58 s at 7.55 mL/s.

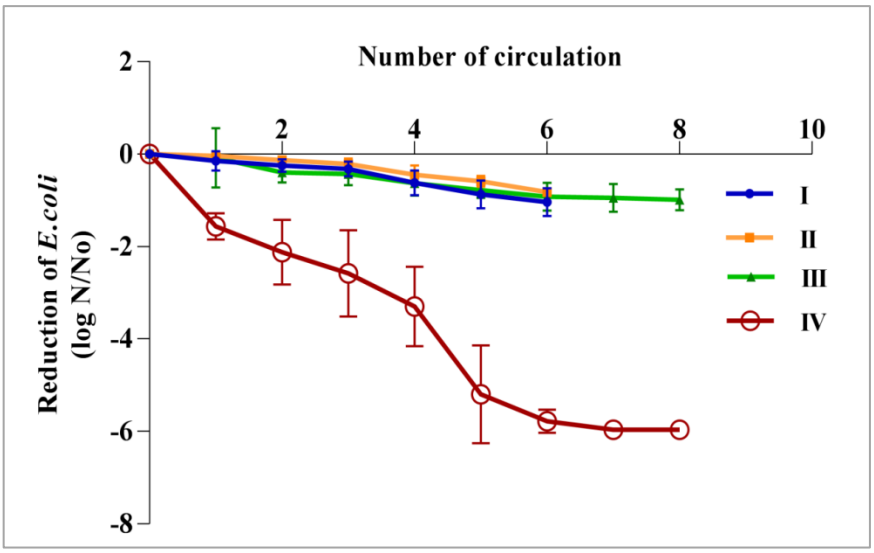


Figure 8.10. Inactivation *of E.coli* K-12 by UV-C treatments
(I: flow rate 3.80 mL/s, 4 lamps on, II: 7.55 mL/s, 4 lamps on; III: 3.80 mL/s, 7 lamps on, IV: 3.80 mL/s, 4 lamps on, assisted with mild heat (55 °C))

8.3.3. UV-C Combined with Mild Heat Processing

Since UV-C irradiation as a stand-alone treatment was not effective to inactivate 5 log of target microorganism, hurdle approach was employed considering combination of UV-C and mild heat. According to the UV-C results, another set of experiment was designed by combining UV design I (3.80 mL/sec flow rate, 4 lamps on) with mild heat (55°C) in order to take advantage of synergic effect of UV-C irradiation and mild heat (Design IV).

UV-C assisted with mild heat treatment resulted in 5.20 ± 1.06 log reduction of *E. coli* K-12 in SJ at the end of 5^{th} cycle. However, it was decided to circulate the juice 6 times in the UV system in order to guarantee the FDA requirement. Circulating the juice 6 times in the system revealed 5.78 ± 0.25 log CFU/mL microbial reduction (Figure 8.10). Hence, it was assured that the 5 log reduction in target microorganism was well satisfied when the juice was circulated 6 times in the system. The total processing time was recorded as 11.3 min at the flow rate of 3.80 mL/s. The total UV-C exposure times (t_{2total}) during 6 cycles of processing were 5.8 min. A picture of samples collected after each cycle of UV-C irradiation can be seen in Figure 8.11. Table 8.1 and Table 8.2 summarize the results obtained from microbial inactivation study in which inoculated strawberry juice samples were subjected to both UV-C and hurdle treatments.

Table 8.1. Logarithmic reduction of *E.coli* K-12 in SJ by UV-C light applied by using different lamp configurations

	UV-C irradiation			**UV-C + MH**
Cycle	I	II	III	IV
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1	0.15 ± 0.21	0.04 ± 0.01	0.08 ± 0.64	1.56 ± 0.29
2	0.25 ± 0.14	0.13 ± 0.04	0.40 ± 0.22	2.12 ± 0.70
3	0.32 ± 0.16	0.21 ± 0.12	0.43 ± 0.24	2.58 ± 0.93
4	0.62 ± 0.27	0.45 ± 0.20	0.63 ±0.27	3.30 ± 0.86
5	0.87 ± 0.30	0.59 ± 0.12	0.78 ± 0.17	5.20 ± 1.06
6	1.04 ± 0.30	0.82 ± 0.00	0.92 ± 0.30	5.78 ± 0.25
7	-	-	0.95 ± 0.30	5.96 ± 0.00
8	-	-	0.99 ± 0.23	5.96 ± 0.00

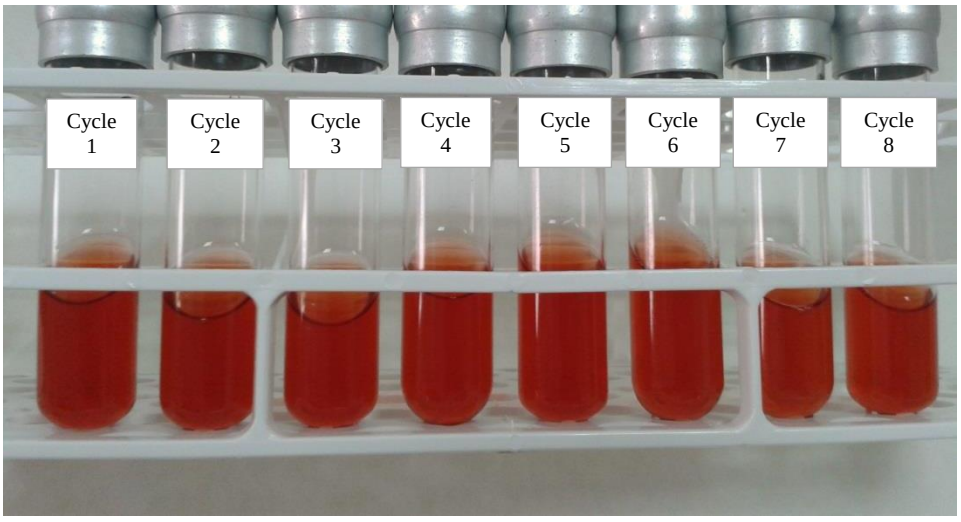


Figure 8.11. Samples collected during 8 cycles of UV-C assisted with MH

Table 8.2. The effect of UV-C and hurdle treatment on the inactivation of *E.coli* K-12 in SJ

Process	**Design**	**Lamp configuration**	**Flow rate (mL/s)**	**UV-C exposure time/cycle (s)**	**Total processing time/cycle (s)**	**Measured UV dose/cycle (J/mL) ***	**Microbial reduction Log(N/N_0)**
UV-C	I	4 lamps on	3.80	58	113	1.77 ± 0.00	1.04 ± 0.30
UV-C	II	4 lamps on	7.55	29	58	1.19 ± 0.00	0.82 ± 0.00
UV-C	III	7 lamps on	3.80	58	113	2.05 ± 0.02	0.92 ± 0.30
UV-C + MH	IV	4 lamps on	3.80	58	113	1.01 ± 0.00	5.78 ± 0.25

(MH refer to mild heat. *Measured UV dose shows the actinometric results.)

Depending on the type of target microorganism and treatment medium, UV-C doses required for 90 % reduction of initial population has been varied from 0.3 to 296 mJ/cm^2 (Gayán et al., 2014). Caminiti et al. (2012) reported UV treatments having at least 2.66 J/cm^2 of exposure dose reduced the counts of *E. coli* below detection limits in apple juice (Caminiti et al., 2012b). Bhat and Stamminger (2015) reported 1 log and 1.5 log reduction in total aerobics and yeast-mold count, respectively, in SJ subjected to static UV treatment with an average UV-C dose of 2.158 J/m^2 for 30 min (Bhat & Stamminger, 2015). A continuous flow UV-C reactor was used for the microbial stability of orange juice by Pala & Toklucu (2013a). The applied UV-C doses varied from 12.03 kJ/L to 48.12 kJ/L (Pala & Toklucu, 2013a). However, the authors theoretically calculated the UV-C dose as a function of total output wattage of the lamps and number of lamps. In the same manner, Gayan el al. (2013) reported the applied UV-C dose for the process where UV-C was combined with mild heating as 27.10 J/mL (Gayan et al., 2013). In the case of this thesis, the applied UV-C dose was estimated by chemical actinometry and the results are presented in Table 8.2. Design I and II, which are constructed considering 4 UV-C lamps, have 1.77 and 1.19 J/mL of UV-C intensity, respectively. UV-C dose was higher when the flow rate was lower. On the other hand, design III with 7 lamps resulted in relatively higher UV-C intensity of 2.05 J/mL. When design I combined with mild heat (design IV), the UV dose reduced to 1.01 J/mL due to the effect of temperature taken into consideration in the calculation of quantum yield in Equation 8.8. Kaya et al. (2015) estimated the applied UV-C dose as 2.461 J/mL based on actinometric measurements (Kaya et al., 2015) which was in line with the findings of this study.

The temperature of the juice increased from 51.40 ± 0.00 °C to 55.05 ± 0.35 °C in the sample tank. Due to the heat loss during circulation of juice, the outlet temperature was lower than that of sample tank. The outlet temperature of strawberry juice treated by the combination of mild heating at 1.01 J/mL of UV-C dose increased from 47.65 ± 0.07 °C to 53.9 ± 0.00 °C after 6 cycles (11.3 min) (Figure 8.12). Geveke (2008) used a tubular UV unit with four UV bulbs in series for liquid egg white pasteurization. The author observed an increase in temperature of the product by 0.5 and 0.1 °C per bulb after UV-C irradiation at the inlet temperature of 30 and 40 °C, respectively. Some other studies in literature applied UV-C irradiation at ambient

temperature (Tran & Farid, 2004) or even less than ambient temperature (8-10 °C) (Keyser, Muller, Cilliers, Nel, & Gouws, 2008).

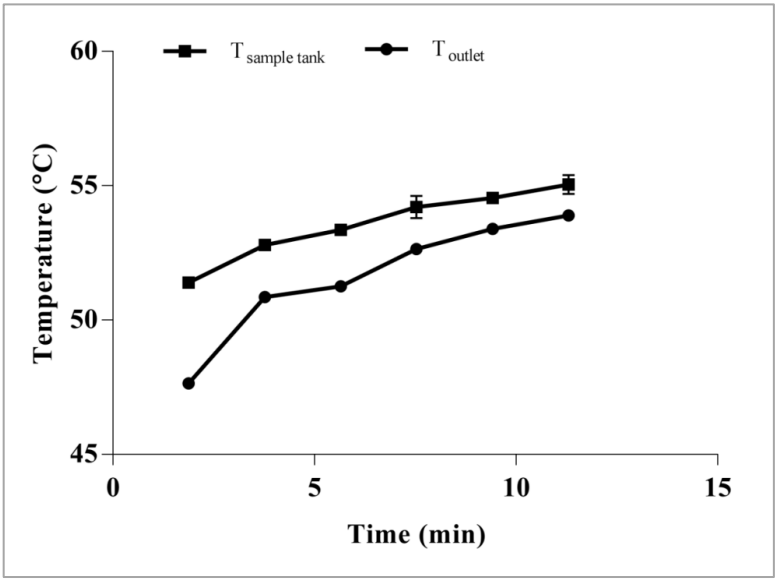


Figure 8.12. Change in temperature during UV-C with mild heat processing (design IV)

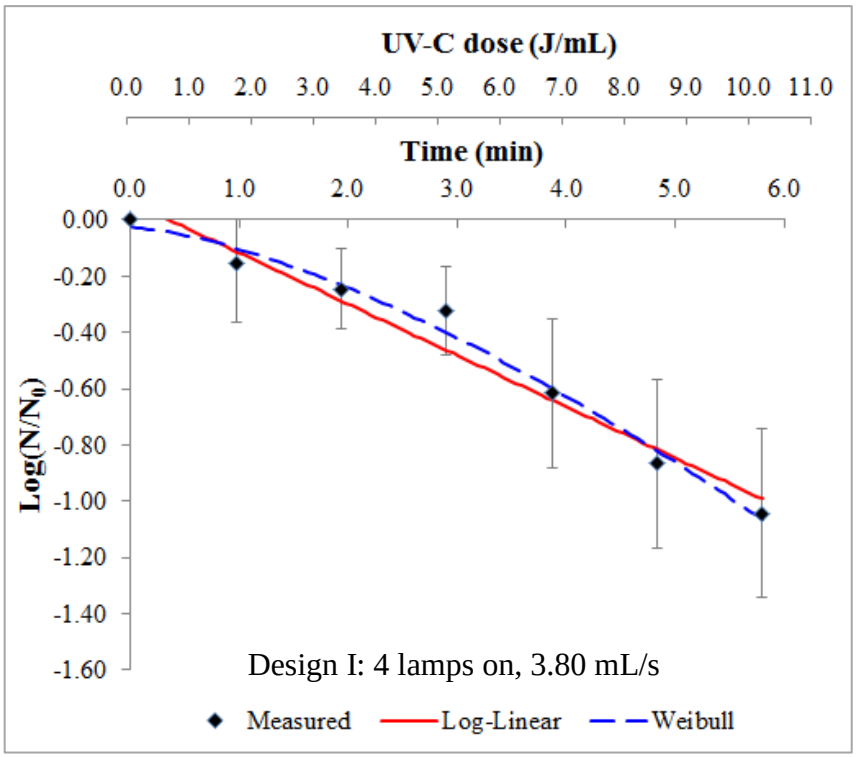
8.3.4. Mathematical Modeling of *E.coli* K-12 Inactivation Data

Log-linear model and Weibull distribution were used for modeling the inactivation kinetics of *E. coli* K-12 in strawberry juice subjected to UV-C irradiation. UV dose response curves of the suspended cells were plotted considering logarithmic reduction versus UV-C exposure times with the corresponding UV-C doses. Figure 8.13, Figure 8.14, and Figure 8.15 show the measured data as well as Log-linear and Weibull models.

Slight concavity trends were observed for design I and design II after applying a UV-C dose of 1.77 and 1.19 J/mL per cycle, respectively. Consequently, design I (4 lamps on; 1.77 J/mL, 3.80 mL/s, 6 cycles, exposure time per cycle: 58 s) and design II (4 lamps on; 1.19 J/mL; 7.55 mL/s; 6 cycles; exposure time per cycle: 29 s) inactivated *E. coli* K-12 by 1.04 ± 0.30 and 0.82 ± 0.00, respectively. Thus, none of the configurations was able to sufficiently inactivate the target microorganism in order to meet FDA's 5 log reduction criterion. Similarly, Design III (7 lamps on, 3. 80 mL/s) reduced the population of *E. coli* K-12 by 0.92 ± 0.30 log (CFU/mL) by UV-C dose of 2.05 J/mL. Log-linear and Weibull model parameters, i.e. k, δ (scale parameter), p

(shape parameter), are given in Table 8.3. k values of *E.coli* K-12 in SJ subjected to varying UV-C doses by design I, II, and III were quite small, and found as 0.42, 0.66, and 0.37 1/min, respectively. Consequently, these small k values resulted in UV-C exposure time of 5.50, 3.51, and 6.18 min for the reduction of 1 logarithmic unit of *E. coli* K-12. When the 5D concept was taken into account, relatively longer processing times would be considered for design I, II, and III. With respect to design I and design II, root mean square error (RMSE) values were higher for Log-linear model (0.0819 and 0.0789, respectively) while Weibull model showed lower RMSE values (0.0562 and 0.0284, respectively). Moreover, shape parameters (p) were greater than 1.0 for design I (1.47) and design II (1.73). These results indicated that application of UV-C irradiation to the strawberry juice containing suspended *E. coli* K-12 resulted in nonlinear inactivation of the target microorganism at UV-C doses of 1.77 and 1.19 J/mL, respectively. Accordingly, the inactivation kinetics of *E. coli* K-12 by design I and II were best described by Weibull model with smaller RMSE and higher R^2 values. Regarding the design III, the shape parameter (0.96) was found close to 1.0 suggesting almost a linear inactivation behavior for *E. coli* with lower RMSE values compared to Weibull model. In any case, both Log-linear and Weibull model would result in quite longer times of UV-C exposure in order to achieve 5 log reduction of *E.coli* K-12 in SJ.

On the other hand, the combination of UV-C light with mild heat treatment (53.9 °C) in design IV resulted in linear inactivation of *E. coli* K-12 in SJ at UV-C dose of 1.01 J/mL per cycle (Figure 8.15). k value was 2.20 1/min which suggested UV-C exposure time of 1.05 min for 1 logarithmic reduction of *E. coli* K-12. The required time to accomplish 5 log reduction of the *E.coli* K-12 in SJ could be estimated as 5.25 min based on Log-linear inactivation model. This finding was actually so close to the time applied for the design IV (5.8 min). The R^2 and RMSE values of Log-linear model for design IV were 0.9643 and 0.4199, respectively. Weibull model was also supported Log-linear model since the shape parameter (1.05) was close 1.0. Although the coefficient of determination (R^2) was high enough for both models, the error of Log-linear model was lower than Weibull distribution. Since the lethal effect was increased by the assistance of external mild heating, design IV (4 UV-C lamps, 1.01 J/mL, 3.80 mL/s, 6 cycle, exposure UV-C time per cycle: 58 s, assisted with 55 °C) achieved 5.78 ± 0.25 log CFU/mL reduction of *E.coli* K-12. Thereby, FDA's 5-log reduction requirement was accomplished. The use of mild heat increased the linearity of inactivation behavior of *E.coli* K-12 in SJ.



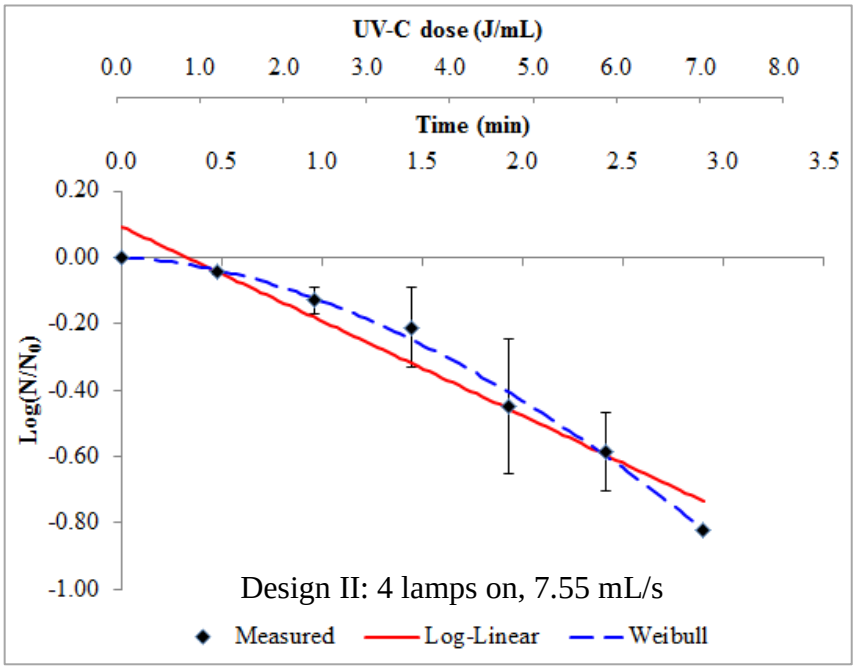


Figure 8.13. Effect of UV-C irradiation treatment using design I and design II on *E.coli* K-12 in SJ

(♦) Experimental values, (-) Log-linear model predicted values, (- -) Weibull model predicted values

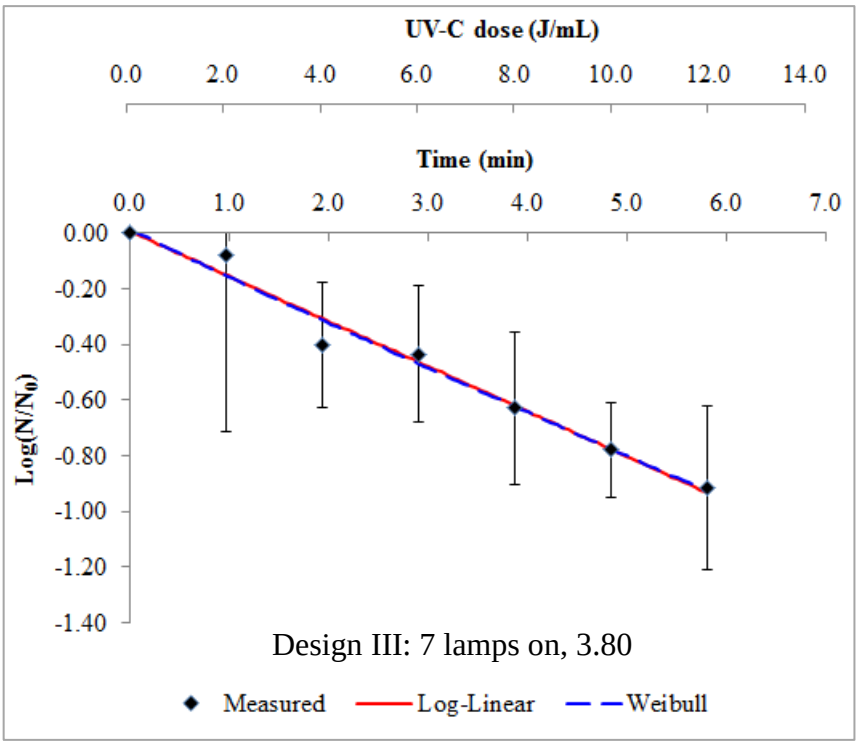


Figure 8. 14. Effect of UV-C irradiation treatment using design III on *E.coli* K-12 in SJ (•) Experimental values, (-) Log-linear model predicted values, (- -) Weibull model predicted values

Inactivation kinetics of *E.coli* K-12 has been modelled in freshly squeezed grape juice by Unluturk & Atılgan (2014). The authors observed higher D values as the flow rate increased. It is claimed that the contact of UV-C light and the target microorganism could be inefficient due to the less UV-C exposure time at higher flow rates (Unluturk & Atilgan, 2014). In accordance with this study, higher levels of *E.coli* K-12 inactivation was achieved in strawberry juice when the flow rate was adjusted to the lower level. The mathematical modeling of inactivation kinetics of *E. coli* K-12 in SJ by UV-C light treatments led to evaluation of resistance of target microorganism and estimation of inactivation behavior considering model parameters such as inactivation rate (k), shape and scale parameters. Considering Log-linear regression model, the highest resistance of *E.coli* K-12 was observed for Design III with a k value of 0.37 1/min. On contrary, design IV resulted in the least resistance of *E.coli* K-12, thereby the highest inactivation with k value of 2.20 1/min (Table 8.3). Weibulian model parameters are also summarized in Table 8.3. Shape parameter p was greater than 1 for design I and II. Design III had a shape parameter below 1 which means that the surviving cells could quickly adapt to exposed lethal agent (Mafart, Couvert, Gaillard, & Leguérinel, 2002) as UV-C dose increased. δ is the scale parameter of Weibull model which is also

defined as the time of first decimal reduction in time unit (min). According to Table 8.3, the time required for the first decimal reduction of *E.coli* K-12 by UV-C with mild heating was 1.05 min. However, the time necessary for 1 logarithmic reduction of the target cells was found as 9.95 min based on Weibull model when the same lamp configuration and flow rate were used in design I where cooling was applied instead of mild heating. Thus, assistance of mild heating enhanced the lethal effect on *E.coli* K-12 suspended in SJ.

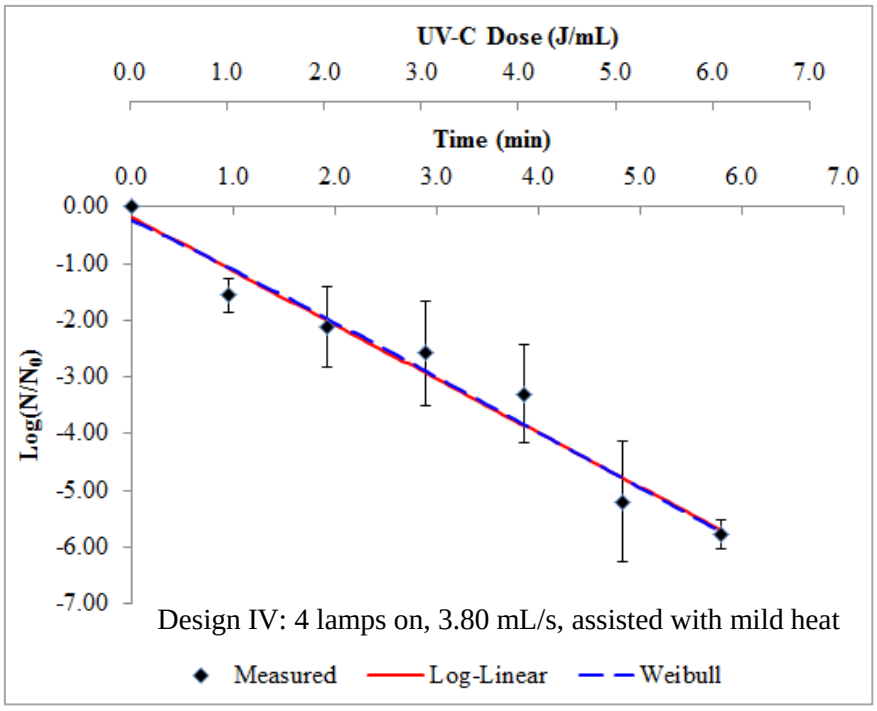


Figure 8.15. Effect of UV-C irradiation and tempreature using design IV on *E.coli* K-12 in SJ

(•) Experimental values, (-) Log-linear model predicted values, (- -) Weibull model predicted values

Table 8.3. Model Parameters for *E. coli* K-12 inactivation in strawberry juice by UV-C treatments

Design	Process	Model	k	p	δ	t (D10)	R^2	RMSE
I	UV-C	Log-Linear	0.42	-	-	5.50	0.9627	0.0819
		Weibull	-	1.47	5.65	9.95	0.9860	0.0562
II	UV-C	Log-Linear	0.66	-	-	3.51	0.9448	0.0789
		Weibull	-	1.73	3.25	5.27	0.9943	0.0284
III	UV-C	Log-Linear	0.37	-	-	6.18	0.9778	0.0557
		Weibull	-	0.96	6.17	14.77	0.9782	0.0617
IV	UV-C + heat	Log-Linear	2.20	-	-	1.05	0.9643	0.4199
		Weibull	-	1.05	1.15	2.53	0.9646	0.4673

k specific inactivation rate [1/min]
p: shape parameter [-]
δ: scale parameter [min]

8.4. Conclusions

In this part of the thesis, the efficacies of conventional thermal, UV-C irradiation and UV-C irradiation under the assistance of external mild heating were evaluated considering *E.coli* K-12, a surrogate of *E.coli* O157:H7, as a target microorganism in strawberry juice. The inactivation behavior of *E.coli* K-12 by the combined effect of thermal and UV-C light was evaluated with respect to the inactivation kinetics. The target inactivation level was at least 5-log CFU/mL in accordance with FDA's 5-log reduction concept. $D_{72\ ^{\circ}C}$ for *E.coli* K-12 in SJ was 20.21 s. Thus, the conditions of thermal pasteurization were determined as 72 ℃ for 101 s due to the achievement of 5 log reduction in target microorganism. UV-C treatments alone were not enough to reach 5-log reduction in inoculated *E.coli* cells in strawberry juice when cold water was circulated through the double walled sample tank. *E. coli* cells showed nonlinear inactivation behavior when SJ subjected to UV-C irradiation alone. Therefore, UV-C irradiation was combined with mild heat in order to increase the lethality. The time required for 1 logarithmic reduction of *E. coli* K-12 was 1.05 min for design IV (4 lamps on; 1.77 J/mL, 3.80 mL/s, 6 cycles, exposure time per cycle: 58 s, mild heating at 53.9 °C). Accordingly, UV-C with mild heating achieved 5.78 ± 0.25 log CFU/mL reduction of *E. coli* in SJ and exhibited linear inactivation behavior. Thus, the results obtained in this chapter were used as a baseline for further UV-C pasteurization of SJ. According to the results of design I, II, and III, it was found that UV-C light treatment was not enough to achieve 5-log reduction in the target microorganism as required by FDA. Since UV-C irradiation as a stand-alone treatment was not effective to inactivate 5 log of target microorganism, hurdle approach was employed considering a combination of UV-C and mild heat.

CHAPTER 9

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

9.1. Introduction

Processing of food products either by thermal or nonthermal technologies has been applied to extend the shelf life and ensure the safety for consumers. Nonthermal processing technologies have been emerged as alternative methods to obtain the final product with desirable sensorial attributes and nutritional characteristics (Falguera, Pagan, Garza, Garvin, & Ibarz, 2011a). Ultraviolet irradiation is one of the nonthermal technologies providing lethal effect on microorganism; thereby reducing the microbial load of the treated medium. It has been widely applied for decontamination of air, surface, and water (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). Recently many scientific studies have been focused on the UV-C light processing of fruit juices such as apple juice (Caminiti et al., 2012b; Gayan, Serrano, Monfort, Alvarez, & Condon, 2013; Müller, Noack, Greiner, Stahl, & Posten, 2014), orange juice (Gayan, Serrano, Monfort, Alvarez, & Condon, 2012; Hakguder Taze, Unluturk, Buzrul, & Alpas, 2015; Pala & Toklucu, 2013a), grape juice (Kaya & Unluturk, 2016; Müller et al., 2014; Pala & Toklucu, 2013b) mango juice (Santhirasegaram, Razali, & Somasundram, 2014), grapefruit juice (La Cava & Sgroppo, 2015), strawberry juice (Bhat & Stamminger, 2015), lemon melon juice blend (Kaya, Yildiz, & Unluturk, 2015).

In this chapter, the microbial stability (total mesophilic aerobic count (TMAC), coliform and yeast-mold (YM) counts) and physicochemical properties such as pH, titratable acidity, total soluble solid (TSS) content, color parameters, i.e. L*(brightness-darkness), a*(redness-greenness), b* (yellowness-blueness), absorption coefficient, and turbidity of freshly squeezed strawberry juice (SJ) pasteurized by a combined UVC-mild heat treatment were monitored during 42 days of refrigerated storage at 4 °C. Besides, phytochemical properties of SJ (total phenolic content (TPC), total anthocyanin content (TAC), antioxidant activity/radical scavenging activity (RSA))

were evaluated immediately after processing. Untreated and thermally pasteurized juice samples were used as negative and positive controls, respectively, for comparison against strawberry juice pasteurized by UVC assisted mild heat treatment.

9.2. Material and Methods

9.2.1. Pasteurization and Storage

Freshly squeezed strawberry juice was prepared using the similar procedure as described in Chapter 4 and Chapter 8. Freshly squeezed SJ was pasteurized by UV-C irradiation and a combined UVC-mild temperature treatment at 53.9 ± 0.00 °C (the water temperature circulated around the juice placed in a holding tank was adjusted to 55 °C). For comparison, some of the SJ samples were also subjected to thermal treatment at 72 °C for 101 s. Regarding UV-C processing; 400 mL of freshly squeezed SJ was initially heated to 52 °C in a beaker containing boiling water (Figure 8.2 in Chapter 8). Then the heated juice was transferred to the sample tank of the UV system (Figure 8.4). Water adjusted to 55 °C was circulated through the walls of the sample tank to provide mild heating. The water around the glass tank was circulated by means of a water bath (Haake DL30, Thermo Electron Corp., Karlsruhe, Germany). 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. SJ was circulated six times through the system using the last configuration (IV) designed for a combined UV-C - mild heating treatment (Figure 8.5). The flow rate at the given system was kept at 3.80 mL/s. Time necessary to complete one cycle of 400 ml SJ in the UV-C system was recorded manually at 3.80 mL/s by using a chronometer. The total processing time and UV-C exposure time per cycle were 113 s and 58 s, respectively. Applied UV-C dose measured by an actinometric method was found as 1.01 ± 0.00 J/mL (Chapter 8, section 8.2.7). The maximum temperatures of SJ at the outlet of UV-C reactor and inside the sample container were 53.9 ± 0.00 °C and 55.05 ± 0.35 °C measured at the end of sixth cycle.

Freshly squeezed strawberry juice was also subjected to thermal treatment and used as positive control in this study. For this purpose strawberry juice placed in a bottle was firstly heated to 70 °C in 5 min a hot plate (Figure 8.2). Then the juice was transferred to the sample tank of the pasteurization unit connected to helical holding tube (see section 8.2.5). Hot water (73 °C) was circulated through the walls of holding tube during processing by placing it into a water bath (Figure 8.3). Previous trials showed that the temperature of juice inside the helical tube reaches to 72 °C when the water bath is at 73 °C. SJ was pumped through the helical holding tube at 13 rpm which corresponded to flow rate of 0.72 mL/s. Thermal pasteurization conditions (i.e. temperature and time parameters) were determined previously by considering the thermal inactivation behavior of *E. coli* K-12 and selected as 72 °C for 101 s. Then, strawberry juice samples subjected to UVC assisted mild heating and thermal treatment, was immediately cooled down in an ice-water bath. Untreated, UV-C+Mild Heat (MH) treated and thermally pasteurized juice samples were then stored in amber color glass bottles at refrigerated conditions (4.0 ± 0.82 °C) for 42 days. Samples were taken at 3-7 days intervals for the analysis. Microbiological analysis (total aerobic bacteria, total coliforms, yeasts and molds) and physicochemical analysis were performed for each sample.

9.2.2. Microbiological Analysis

Microbial quality of strawberry juice samples before and after processing was evaluated in terms of total mesophilic aerobic bacteria count (TMAC), yeasts-molds (YM), and coliform bacteria count The samples were plated on Plate Count Agar (PCA), Violet Red Bile Agar (VRBA) and Potato Dextrose Agar (PDA) acidified with 10 % of tartaric acid by spread plating method for TMAC, coliform and YM count, respectively. The plates were then incubated at 30 °C for 48 h for TMAC, 37 °C for 18 h for coliform and 25 °C for 5 days for YM count. After appropriate time of incubation, the number of viable cells was counted, and the results were expressed as colony forming units (CFU) per milliliter (Tran & Farid, 2004). Untreated SJ was used as negative control.

9.2.3. Measurement of Physicochemical and Phytochemical Properties

Physicochemical properties such as pH, titratable acidity, total soluble solid (TSS) content, color parameters, i.e. L*(brightness-darkness), a*(redness-greenness), b* (yellowness-blueness), absorption coefficient, and turbidity of strawberry juice samples before and after pasteurization were measured according to the methods described in Chapter 4. Briefly, pH was measured at 22°C using a bench top pH meter (inoLab pH 7310, WTW GmbH, Weilheim, Germany). Titratable acidity was determined based on NaOH titrimetric method. Results were expressed as g citric acid/100 mL. A digital hand-held pocket refractometer (Atago CO., LTD.) was used to determine the total soluble solids (°Brix) of the samples. CIE L*, a*, b* values of the samples (30 mL) were measured by a Chroma meter (CR 400, Konica Minolta Inc., Japan). Then, total color difference (ΔE) and browning index values were calculated based on the equations given in Chapter 4. Absorption coefficient of SJ was determined spectrophotometrically by measuring the absorbance of different dilutions of SJ samples at 254 nm using quartz cuvettes with a path length of 1 cm. Absorbance versus concentration plot was drawn in order to calculate the absorption coefficient from the slope of the plot. Turbidity was measured through a turbidimeter, and the results were given in terms of Nephelometric Turbidity Unit (NTU).

Phytochemical properties of strawberry juice before and after pasteurization were evaluated with respect to total phenolic content (TPC), total anthocyanin content (TAC), antioxidant activity/radical scavenging activity (RSA). Details of the analytical assays can be found in Chapter 4. Concisely, TPC of strawberry juice were determined according to Folin-Ciocalteu method (Singleton & Rossi, 1965) modified by Tezcan et al, (Tezcan, Gultekin-Ozguven, Diken, Ozcelik, & Erim, 2009). Results were expressed as gallic acid equivalents (GAE). A modified pH differential method described by Meyers et al, (Meyers, Watkins, Pritts, & Liu, 2003) was used for the determination of total monomeric anthocyanin content of strawberry juice. TAC of SJ was expressed as mg pelargonidin-3-glucoside/L. Antioxidant activities of strawberry juice samples were measured with a stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), and expressed as % radical scavenging activity (Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2009).

9.2.4. Data Analysis

Pasteurization of SJ by thermal and UVC-mild heat treatment was conducted in duplicate. Microbiological assays and physicochemical analysis were also performed in duplicate. Phytochemical properties of SJ samples were carried out in triplicate. One-way analysis of variance (ANOVA) was used in order to determine how significantly the independent variables (treatments or storage days) affect the changes in the dependent variables. The means were compared by ANOVA considering Tukey's comparison test in 95 % of confidence interval. Data analyses were carried out by Minitab 16 (Minitab Inc., State College, PA, USA) and a data sheet (Microsoft Excel, 2010). Results were expressed as means ± standard deviation. Moreover, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to visualize the data structure and distinguish similarities and differences among treatments and storage days considering physicochemical attributes of SJ simultaneously. The details of PCA and HCA are given in Chapter 6, section 6.2.3.

9.3. Results and Discussion

9.3.1. Microbiological Quality of Strawberry Juice after Processing and During Storage

Logarithmic changes in total mesophilic aerobic bacteria (TMAC), yeast and mold (YM) counts and coliform counts of SJ samples which are untreated (control), treated with UV-C+mild heating and thermally pasteurized, during 42 days of refrigerated storage (4.0 °C) were evaluated. Strawberries are highly susceptible to microbial spoilage due to their soft skin and nutritional composition (Tournas, Heeres, & Burgess, 2006). The initial microbial counts in SJ were 3.36 ± 0.04 log CFU/mL of TMAC, 3.87± 0.07 log CFU/mL of YM and 2.26 ± 0.01 log CFU/mL of coliforms, respectively (Figure 9.1). According to the microbial criteria mandated by Turkish Food Codex (2002) (Microbiological Criteria, No: 2001/19) and practical working guidance provided by Institute of Food Science and Technology (IFST, 1999), the acceptable

maximum TMAC and YM counts in fruit juice and nectars must be 4 and 3 log CFU/mL, respectively. These limits were taken into consideration as microbial criteria for the shelf life evaluation of SJ pasteurized using UV-C+mild heat treatment and thermal treatment. The juice had initially >3 log CFU/mL YM count which was higher than this microbial criteria.

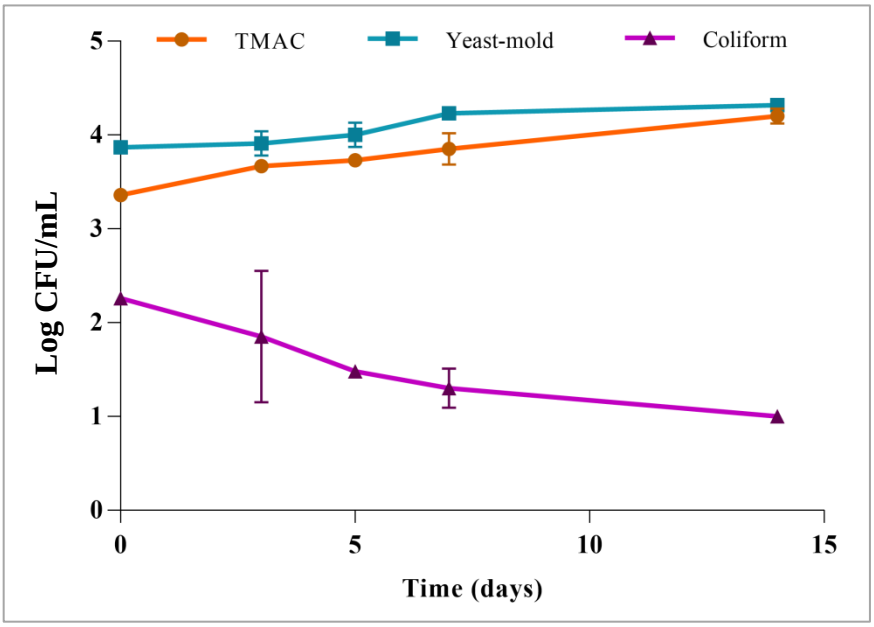


Figure 9.1. The effect of storage (4°C for 15 days) on the natural flora of untreated strawberry juice

UV-C treatment assisted with mild heating (UV-C+MH) were able to increase the lethal effect on natural flora and ensured the microbial safety of SJ. Upon UV-C+MH treatment (1.01 J/mL, 6 cycles, 5.8 min, 3.80 mL/s, 53.9 °C) and thermal pasteurization (72 °C, 101 s), TMAC, YM, and coliform counts in SJ were reduced below the detectable limit. In other words, no viable cells in treated SJ were detected until the end of storage period. At the similar processing conditions, at least 5 log reduction was achieved in the number of *E.coli* K-12 inoculated in SJ (see Chapter 8). Pala and Toklucu (2013a) also reported 5.72 log reduction of *E. coli* ATCC 25922 after UV-C treatment (36.09 kJ/L) while the same treatment resulted in 2.8 log and 0.34 log reduction in TMAC and YM count in orange juice (Pala & Toklucu, 2013a). Char et al. (2006) also showed that UV-C treatment provided lower inactivation in yeast compared to *E.coli* (ATCC 35218) (Char, Mitilinaki, Guerrero, & Alzamora, 2010). Less susceptibility of yeasts and molds to UV-C light compared to bacteria could be aroused

from the difference in the thickness of cell wall and size of microorganism which have influence on transfer of UV-C light. Moreover, lesser pyrimidine bases on the DNA strand of yeast and mold correspond to less probability of cross link formation, thus higher resistance to UV-C light (Miller, Jeffrey, Mitchell, & Elasri, 1999). However, the UV-C dose studied in Pala and Toklucu (2013a) was higher (36.09 J/mL) than that of applied (1.01 J/mL) in this study. Besides, no viable cells were detected in treated SJ in contrast to the findings of Pala and Toklucu (2013a). The reason of this was due to the combination of UVC irradiation with mild heating providing higher lethal effect on microorganisms.

UV treatment combined with mild temperature (50-60 °C) has been reported to create synergistic lethal effect on yeasts. (Gouma, Gayán, Raso, Condón, & Álvarez, 2015) achieved 5 log reduction of *S. cerevisiae* in apple juice by combining UVC with mild heat (Gouma et al., 2015). Gayan et al. (2012) also combined UV-C light (13.55 J/mL) with mild heat treatment at varying temperatures up to 60 °C in order to increase the effectiveness of the UV treatment for the pasteurization of orange juice. The authors observed a synergistic effect when both treatments were applied simultaneously and they stated that the maximum lethal effect was achieved at 55 °C (Gayan et al., 2012). Synergistic effect on microbial inactivation can be determined with respect to the mode of action of each individual treatment (Gouma et al., 2015). However, the inactivation data was not modelled in the present study. Thus it would be difficult to sharply address the whether the inactivation effect of processes are synergistic or additive. Nonetheless, the reductions in TMAC, YM and coliform count obtained by UV-C+MH were attributed to the synergistic effect of combined treatments. Synergistic effects occur due to the sub-lethal damage on various cellular targets by different processing technologies (Leistner, 2000). Accordingly, synergistic inactivation of yeasts by UV-C irradiation and mild heat (45–60 °C) has been attributed to the impairment of its DNA-damage repair ability (Petin, Kim, Rassokhina, & Zhurakovskaya, 2001).

Since fresh juice (untreated juice) is a suitable medium for the microbial growth (Tournas et al., 2006), the YM count increased to 4.32 ± 0.07 log CFU/mL at the end of 14 days under refrigeration conditions (4 °C) (Figure 9.1). Pala and Toklucu (2013a) addressed the yeast and mold count as the mainly deteriorating factor for orange juice during shelf life. Besides, the authors realized a rapid increase in total aerobic count of UV-C treated orange juice during storage (Pala & Toklucu, 2013a). Regarding the total

aerobic bacteria count of untreated SJ in this study, the initial population reached to 4.20 ± 0.08 log CFU/mL at the end of refrigerated storage of 15 days which was higher than the microbial limit (4 log CFU/mL) mandated by Turkish Food Codex (Codex, 2001). No viable cells were detected in UV-C+MH treated SJ samples during subsequent refrigerated storage up to 42 days. In the current study, the shelf life of SJ treated with UV-C+MH had a much longer shelf life compared to UV-C treated white grape juice studied by Unluturk and Atılgan (2015). The authors processed grape juice by an annular flow UV system at 0.90 mL/s for 32 min by circulating 8 times through the system, and finally prolonged the shelf life of white grape juice up to 14 days (Unluturk & Atilgan, 2015). Likewise, UV-C treated orange juice had a shelf life of 9 and 5 days at 4 and 10 °C (Pala & Toklucu, 2013a), respectively that is lower compared to the shelf life of SJ obtained in this study. Tran and Farid (2004) evaluated the shelf life of UV-C treated orange juice considering a microbial criterion as 5000 CFU/mL. Thus, shelf life of orange juice was extended more than 5 days and proposed to be prolonged further by applying higher UV-C doses (Tran & Farid, 2004).

Al-Jedah and Robinson (2002) reported that coliform bacteria count permitted in fruit juices must be maximum 100 CFU/mL (2 log CFU/mL). In this study, the initial load of total coliforms in untreated SJ was 2.26 ± 0.01 log CFU/mL and decreased below 2 log CFU/mL during 14 days of storage at 4 °C (Figure 9.1). The decrease in the number of coliform population was attributed to the chilling injury of some mesophilic bacteria during storage (Tran & Farid, 2004). Kaya et al. (2015) also observed a decrease in total coliform count of untreated lemon melon juice blend (LMJ) during storage period. They reported the initial coliform count in LMJ as 2.21 ± 0.17 log CFU/mL and 1.85 ± 0.13 log CFU/mL at the end of storage period (5 days). Their findings support the results of this thesis. In the contrary, Müller et al. (2014) found the total aerobic count of both untreated and UV-C treated apple juices to be > 7 log CFU/mL at the end of storage. On the other hand, they reported YM counts of untreated and UV-C treated juices to be approximately 5 log CFU/mL(Müller et al., 2014). Nonetheless, UV-C irradiation has been successfully applied to different juice product to accomplish a reduction of microorganisms due to its germicidal effect at 254 nm (Gayán, Condón, & Álvarez, 2014; Keyser, Muller, Cilliers, Nel, & Gouws, 2008; Koutchma, 2009b; Koutchma, Popovic, Ros-Polski, & Popielarz, 2016). Moreover,

UV-C light treatment provides advantages including reduced cost, simple and low maintenance (Guerrero-Beltran & Barbosa-Canovas, 2004).

Likewise, no microbial growth was observed for thermally pasteurized SJ juice at 72 °C for 101 s and stored during 42 days at 4 °C. This finding is in accordance with other studies related to mild heat pasteurization of fruit juices cited in the literature. For instance, Timmermans et al (2011) applied mild heat pasteurization at 72 °C for 20 s for orange juice, and consequently reduced total aerobics and yeast-mold count below detection limits (Timmermans et al., 2011). Kaya et al. (2015) subjected lemon melon juice blend to mild pasteurization at 72 °C for 71 s, and thereafter ensured the microbial safety during 30 days of refrigerated storage (Kaya et al., 2015). Besides, higher temperatures have been also applied for pasteurization of fruit juices. Walkling et al. (2009) subjected orange juice to high temperature short time pasteurization at 94 °C for 26 s, and accomplished microbial safety during 168 days of storage at 25 °C by keeping the microbial load within safe limits (less than 3 log CFU/mL). Thermal pasteurization of mango juice at 90 °C for 60 s also achieved complete inactivation of aerobic bacteria, yeast-molds, and coliforms (Santhirasegaram, Razali, George, & Somasundram, 2015a).

9.3.2. Physicochemical Properties of Strawberry Juice after Processing and During Storage

Physicochemical properties of products such as chemical composition, total soluble solids (TSS), and color properties have been indicated as the important factors affecting both the absorption and effectiveness of UV processing (Koutchma et al., 2016). Changes in physicochemical properties of SJ after processing as well as during storage period are given in Table 9.1 and Table 9.2. Variance analysis (ANOVA) with Tukey comparison test showed the significant differences considering 95 % of confidence interval. Therefore, different super subscript letters on left side of the data obtained at day 0 indicate the significant differences among the product immediately after processing. Different capital, small case, italic small case letters in the same column refer to the significant differences of each product on the basis of storage days.

Table 9.1. The effect of treatments and storage on the physicochemical properties of strawberry juice

Day	Process	pH	TSS (°Brix)	TA (mg/100 mL)	Turbidity (NTU)	Abs. Coeff. (1/cm)
0	Control	[a]3.52 ± 0.00A	[a]5.79 ± 0.19A	[a]0.59 ± 0.01A	[a]144.25 ± 30.05A	[a]35.42 ± 0.82A
	Thermal	[a]3.53 ± 0.00a	[a]5.73 ± 0.07a	[a]0.58 ± 0.00a	[a]161.25 ± 33.59a	[a]35.74 ± 1.08a
	UV+MH	[a]3.53 ± 0.00*a*	[a]5.80 ± 0.07*a*	[a]0.58 ± 0.00*b*	[a]154.75 ± 30.05*a*	[a]34.11 ± 0.58*a*
3	Control	3.51 ± 0.00AB	5.77 ± 0.09A	0.59 ± 0.01A	145.50 ± 29.70A	35.25 ± 1.05A
5	Control	3.51 ± 0.00AB	5.64 ± 0.09A	0.59 ± 0.01A	147.75 ± 31.47A	34.79 ± 1.05AB
7	Control	3.51 ± 0.00AB	5.60 ± 0.00A	0.59 ± 0.00A	151.25 ± 30.76A	34.44 ± 0.20AB
	Thermal	3.53 ± 0.00a	5.63 ± 0.04a	0.58 ± 0.00a	161.50 ± 37.48a	33.74 ± 0.01ab
	UV+MH	3.52 ± 0.00*ab*	5.60 ± 0.00*a*	0.58 ± 0.00*b*	170.50 ± 44.55*a*	33.23 ± 0.05*a*
14	Control	3.49 ± 0.01AB	5.58 ± 0.11A	0.60 ± 0.00A	155.50 ± 32.53A	33.29 ± 0.26AB
	Thermal	3.52 ± 0.00a	5.60 ± 0.00a	0.58 ± 0.00a	166.00 ± 37.48a	32.87 ± 1.47ab
	UV+MH	3.52 ± 0.00*ab*	5.70 ± 0.14*a*	0.59 ± 0.00*ab*	172.75 ± 49.85*a*	29.80 ± 1.47*b*
21	Control	3.49 ± 0.01B	5.58 ± 0.04A	0.60 ± 0.01A	157.83 ± 29.46A	31.90 ± 0.49B
	Thermal	3.52 ± 0.00a	5.60 ± 0.00a	0.59 ± 0.00a	166.75 ± 34.29a	31.71 ± 0.24b
	UV+MH	3.52 ± 0.00*ab*	5.65 ± 0.07*a*	0.59 ± 0.00*ab*	172.50 ± 48.08*a*	28.91 ± 0.27*bc*
28	Thermal	3.52 ± 0.00a	5.68 ± 0.04a	0.59 ± 0.00a	184.00 ± 44.55a	27.29 ± 1.36c
	UV+MH	3.51 ± 0.01*b*	5.65 ± 0.07*a*	0.60 ± 0.01*a*	176.50 ± 48.79*a*	27.23 ± 0.94*bc*
35	Thermal	3.52 ± 0.01a	5.68 ± 0.04a	0.59 ± 0.00a	188.75 ± 44.19a	26.80 ± 0.49c
	UV+MH	3.51 ± 0.00*b*	5.65 ± 0.07*a*	0.60 ± 0.00*a*	181.25 ± 52.68*a*	25.99 ± 0.84*c*
42	Thermal	3.52 ± 0.00a	5.65 ± 0.07a	0.59 ± 0.00a	190.75 ± 42.78a	27.38 ± 1.00c
	UV+MH	3.52 ± 0.01*ab*	5.65 ± 0.07*a*	0.60 ± 0.00*a*	181.58 ± 48.20*a*	26.23 ± 0.88*c*

Results were expressed as mean ± std. Different super subscript letters on left side of the data indicate the significant differences among treatments at day 0 while capital, small case, italic small case letters in the same column refer to the significant changes among storage days of each product ($p<0.05$). (TSS:Total soluble solid, TA: Titratable acidity, Abs. Coeff: Absorption coefficient).

Table 9.2. The effect of treatment and storage on the color properties of strawberry juice

Day	Process	L*	a*	b*	ΔE	Browning Index
0	Control	[a]20.45 ± 0.03A	[a]9.48 ± 0.22A	[a]5.04 ± 0.14A	[b]0.00 ± 0.00B	[a]60.28 ± 1.52A
	Thermal	[b]20.12 ± 0.06a	[a]8.95 ± 0.31a	[a]4.56 ± 0.18a	[a]0.78 ± 0.10c	[a]56.42 ± 1.98ab
	UV+MH	[ab]20.36 ± 0.07*a*	[a]9.13 ± 0.45*a*	[a]4.85 ± 0.24*a*	[ab]0.40 ± 0.26*a*	[a]58.22 ± 2.78*a*
3	Control	20.44 ± 0.01A	9.44 ± 0.22A	5.04 ± 0.08A	0.06 ± 0.00B	60.21 ± 1.27A
5	Control	20.37 ± 0.02AB	9.53 ± 0.29A	5.00 ± 0.12A	0.11 ± 0.04B	60.40 ± 1.63A
7	Control	20.33 ± 0.01B	9.38 ± 0.35A	4.85 ± 0.12A	0.26 ± 0.03AB	59.06 ± 1.86A
	Thermal	20.21 ± 0.11a	8.96 ± 0.40a	4.69 ± 0.22a	0.67 ± 0.21c	57.06 ± 2.44a
	UV+MH	20.46 ± 0.06*a*	9.20 ± 0.42*a*	5.02 ± 0.21*a*	0.28 ± 0.20*a*	59.27 ± 2.55*a*
14	Control	20.34 ± 0.04AB	9.34 ± 0.18A	4.88 ± 0.10A	0.24 ± 0.04AB	59.14 ± 1.16A
	Thermal	20.09 ± 0.06a	8.74 ± 0.35a	4.51 ± 0.17ab	0.97 ± 0.12bc	55.53 ± 2.05ab
	UV+MH	20.40 ± 0.03*a*	9.05 ± 0.26*a*	4.92 ± 0.02*a*	0.46 ± 0.00*a*	58.29 ± 1.03*a*
21	Control	20.34 ± 0.04AB	9.10 ± 0.03A	4.72 ± 0.02A	0.50 ± 0.21A	57.35 ± 0.09A
	Thermal	20.14 ± 0.05a	8.69 ± 0.11a	4.54 ± 0.01ab	0.98 ± 0.18bc	55.40 ± 0.54ab
	UV+MH	20.42 ± 0.09*a*	9.19 ± 0.40*a*	4.92 ± 0.23*a*	0.31 ± 0.20*a*	58.64 ± 2.52*a*
28	Thermal	20.04 ± 0.05a	8.24 ± 0.34a	4.20 ± 0.15ab	1.55 ± 0.11ab	52.00 ± 1.96ab
	UV+MH	20.41 ± 0.10*a*	9.05 ±0.54*a*	4.82 ± 0.29*a*	0.49 ± 0.36*a*	57.57 ± 3.36*a*
35	Thermal	20.19 ± 0.13a	8.41 ± 0.38a	4.44 ± 0.18ab	1.25 ± 0.18bc	53.70 ± 2.06ab
	UV+MH	20.58 ± 0.19*a*	9.29 ± 0.48*a*	5.18 ± 0.42*a*	0.40 ± 0.03*a*	60.24 ± 3.74*a*
42	Thermal	19.91 ± 0.06a	7.86 ± 0.20a	3.95 ± 0.00b	2.02 ± 0.11a	49.46 ± 0.85b
	UV+MH	20.36 ± 0.06*a*	8.87 ± 0.41*a*	4.80 ± 0.25*a*	0.66 ± 0.22*a*	57.01 ± 2.75*a*

Results were expressed as mean ± std. Different super subscript letters on left side of the data indicate the significant differences among treatments at day 0 while capital, small case, italic small case letters in the same column refer to the significant changes among storage days of each product ($p<0.05$). (L*: lightness-darkness, a*:redness-greenness, b*:yellowness-blueness, ΔE: Total color difference, BI: browning index)

pH of untreated strawberry juice was measured as 3.52 ± 0.00 which was similar to that of SJ used for the studies conducted at WSU (see Chapter 5-7). Neither thermal pasteurization nor UV-C+MH treatment altered the pH of SJ after processing ($p>0.05$). No significant change was observed for the thermally pasteurized and UV-C+MH treated SJ throughout storage as well (Table 9.1 and Figure 9.2).

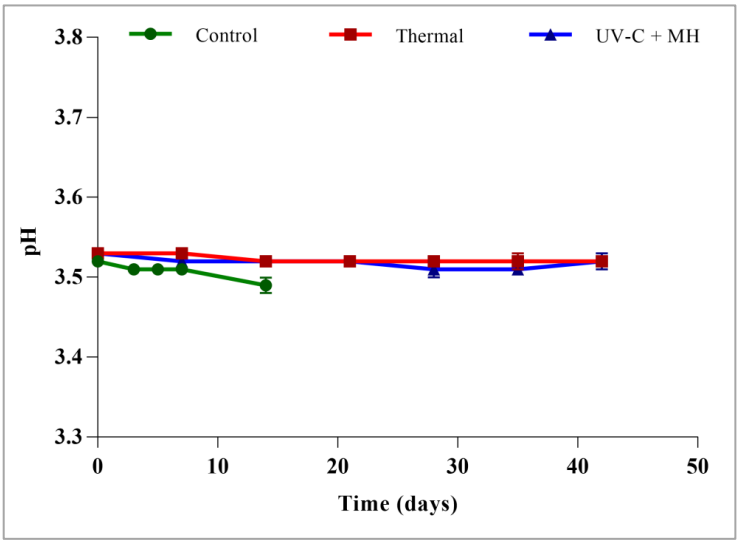


Figure 9.2. The effect of storage on pH value of strawberry juice subjected to different treatments
(Control, Thermal, UV-C+MH refer to untreated, thermal pasteurization at 72 °C, and UV-C assisted with mild heat treatment)

Figure 9.3 depicts the effect of storage on the titratable acidity (TA) of SJ samples subjected to thermal and UVC+MH treatments. TA values expressed in terms of mg citric acid/100 mL are also given in Table 9.1. TA values of SJ treated with UV-C+MH heating varied from 0.58 ± 0.00 to 0.60 ± 0.00 until the end of 42nd day. Similar to pH, no significant difference was observed among TA values of untreated and treated juice samples at the processing day ($p>0.05$). Processed SJ did not show any significant change during storage of 42 days ($p>0.05$).

The initial TSS of untreated SJ was measured as 5.79 ± 0.19 which was lower than that of SJ used for studies conducted at WSU (see Chapter 5-7) (Table 9.1). The TSS content of different juice products e.g. apple juice (11.2 °Brix) (Caminiti et al., 2012b), mango juice (14.7 °Brix) (Santhirasegaram et al., 2015a), white grape juice (18.6 °Brix) (Unluturk & Atilgan, 2015) were also reported to be higher than this measured value. Soluble content of fruit juices could be changed depending on the

characteristics of the juices of raw material, mechanical extraction processes (Codex, 2005). In this case, no significant differences were observed between TSS values of untreated and treated SJ samples at day 0 and during storage (Table 9.1 and Figure 9.4). Even though TSS of untreated SJ slightly decreased during 14 days of refrigerated storage period, the decrement was not statistically significant ($p>0.05$).

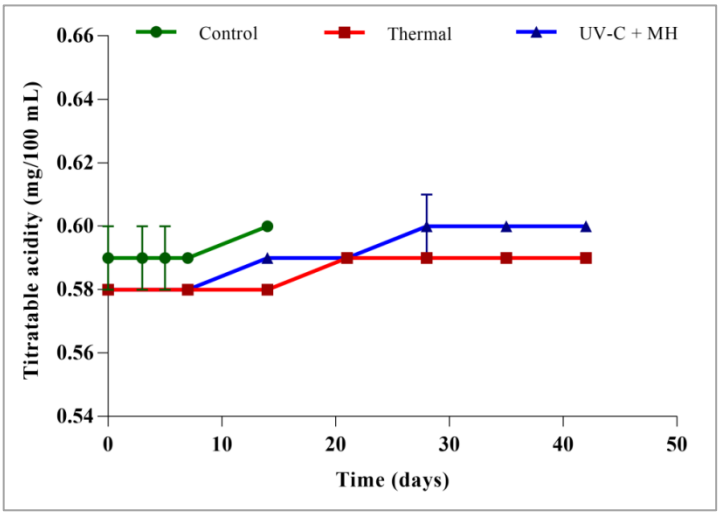


Figure 9.3. The effect of storage on the titratable acidity of strawberry juice subjected to different treatments
(Control, Thermal, UV-C+MH refer to untreated, thermal pasteurization at 72 °C, and UV-C assisted with mild heat treatment)

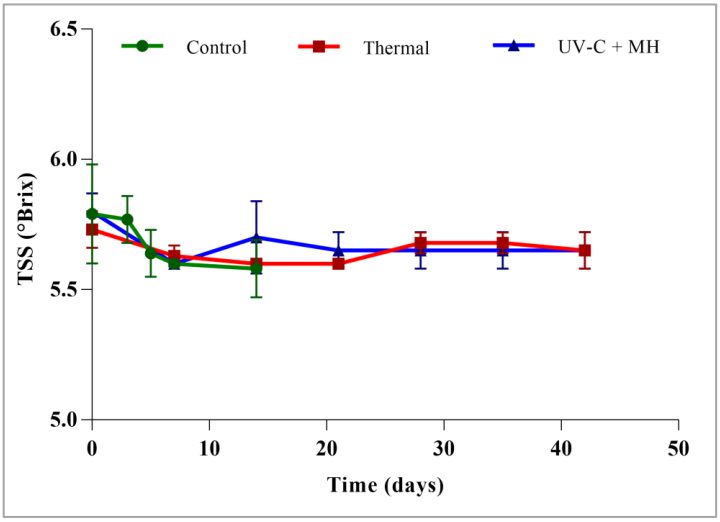


Figure 9.4. The effect of storage on the total soluble content of strawberry juice subjected to different treatments
(Control, Thermal, UV-C+MH refer to untreated, thermal pasteurization at 72 °C, and UV-C assisted with mild heat treatment)

The findings of this work were in good agreement with the results of studies cited in the literature. For example, physicohemical properties (pH, TSS and TA) of different juice products such as apple juice (Walkling-Ribeiro et al., 2008), pomegranate juice (Pala & Toklucu, 2011), orange juice (Pala & Toklucu, 2013a), grape juice (Pala & Toklucu, 2013b), mango juice (Santhirasegaram et al., 2015a) subjected to thermal pasteurization or UV-C light treatment were not affected from the type of treatments and they were not significantly changed after processing.

Nonsoluble solid content of fruit juices have been considered to be important for the efficacy of UV-C irradiation since the light transmission through the medium would be influenced by the transparency or turbidity of the juice (Guerrero-Beltran & Barbosa Cánovas, 2011b). Greater turbidity of the liquid could result in greater the absorption coefficient which means less penetration of light through the system (Guerrero-Beltran & Barbosa-Canovas, 2004). In other words, UV-C irradiation would have more influence and lethal effect for clear fruit juices since suspended particles could absorb, scatter, or block UV light in turbid juices (Christenen & Linden, 2001). Therefore, turbidity is a critical parameter used for estimation of applied UV dose (Koutchma et al., 2016). Kaya and Unluturk, (2016) reported the turbidity of clear and turbid grape juices as 32.5 and 105 NTU, respectively (Kaya & Unluturk, 2016). No clarification was applied in the case of SJ in the current study. Thus, the turbidity of untreated SJ was 144.25 ± 30.05 NTU which was relatively higher than that of turbid grape juice reported by Kaya and Unluturk, (2016). None of the treatments caused any significant change in turbidity of SJ right after processing. Moreover, no significant changes were observed for the turbidity of SJ during refrigerated storage (Figure 9.5, Table 9.1). On the contrary, Tandon et al. (2003) pointed out a significant increase in turbidity during storage of UV treated apple cider (Tandon, Worobo, Churey, & Padilla-Zakour, 2003). 2003. Any changes occurred in turbidity of processed juices reported in literature can be attributed to the protein and polyphenol complexes (Lee, Yusof, Hamid, & Baharin, 2007) or can be related to the residual PME activity after processing (Rivas, Rodrigoa, Martíneza, Barbosa-Cánovas, & Rodrigo, 2006) .

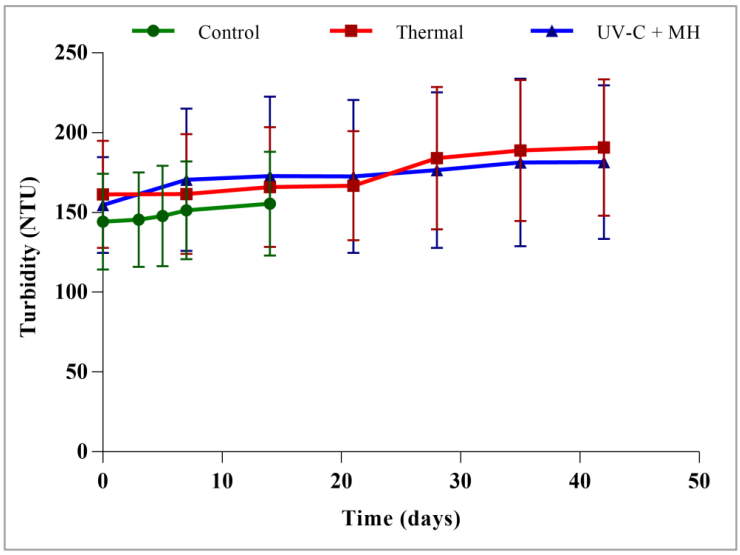


Figure 9.5. The effect of storage on turbidity of strawberry juice subjected to different treatments
(Control, Thermal, UV-C+MH refer to untreated, thermal pasteurization at 72 °C, and UV-C assisted with mild heat treatment)

Absorption coefficient is an indicator of transmittance of UV light through the treatment medium. Composition of fruit juice, i.e. pigments, sugars, organic acids, and suspended particles, can affect the absorption and reduce the transmission of UV-C light; thus, performance of UV-C treatments can be lowered (Koutchma et al., 2016). For instance, absorption coefficient of orange juice (47.9 1/cm) was reported to be higher than that of apple juice (25.9 1/cm) due to its pulp content and turbidity (Koutchma, Paris, & Patazca, 2007). The initial absorption coefficient of untreated SJ was 35.42 ± 0.82 1/cm which was found in the range of that of several juices varying from 12.3 to 52.4 1/cm (Koutchma et al., 2016). Absorption coefficient of SJ was relatively close to that of grape juice (36.5 1/cm) (Unluturk & Atilgan, 2014). High amount of solids or suspended particles would diminish the intensity of penetration of UV-C light and block the lethal effect of light on microorganisms (Bintsis et al., 2000). Therefore, UV-C treatments are providing more advantages for clear liquids. In case of turbid juice, a higher UV dose would be necessary to be applied to the fruit juice in order to accomplish similar log reductions (Unluturk & Atilgan, 2015). Figure 9.6 shows the change in absorption coefficient of SJ samples during refrigerated storage. Unlike other physicochemical parameters, absorption coefficient of SJ samples showed some significant differences during storage period. Irrespective of treatment type, absorption coefficient of all SJ samples tended to decrease as the storage time increased.

A similar trend was also observed for heat and UV-C treated lemon melon juice blend during 30 days of storage (Kaya et al., 2015). The turbidity changes during storage can be related with the pectin content of the juice. It was reported that the turbidity loss rate was reversely proportional to the concentration of pectin (Mirhosseini et al., 2008).

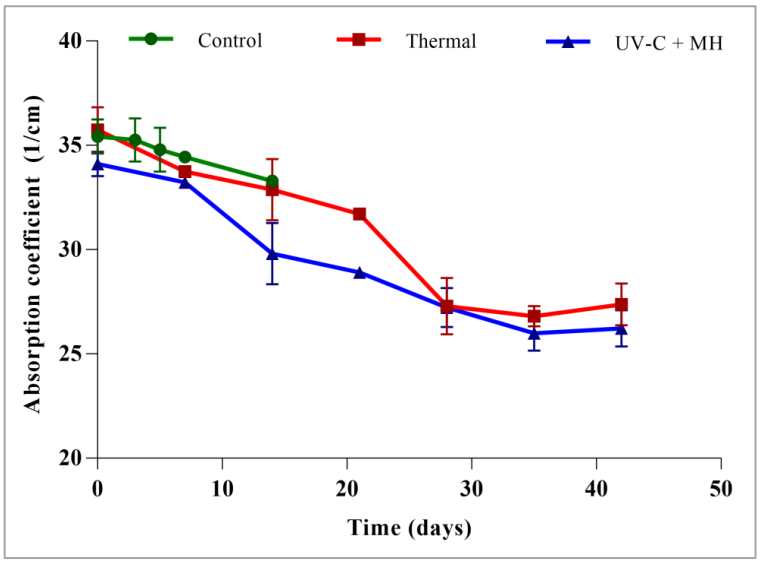


Figure 9.6. The effect of storage on the absorption coefficient of strawberry juice subjected to different treatments
(Control, Thermal, UV-C+MH refer to untreated, thermal pasteurization at 72 °C, and UV-C assisted with mild heat treatment)

The effect of treatments and storage on the color attributes of SJ, i.e. L* (lightness-darkness), a* (redness-greenness), b* (yellowness-blueness), total color difference (ΔE), and browning index (BI), is summarized in Table 9.2 and viewed in the picture provided in Figure 9.7. The initial L*, a*, and b* values of untreated SJ were 20.45 ± 0.03, 9.48 ± 0.22, 5.04 ± 0.14, respectively. UV-C+MH treated SJ had L*, a*, b* values of 20.36 ± 0.07, 9.13 ± 0.45, 4.85 ± 0.24, respectively. On the other hand, thermal pasteurization had L*, a*, b* values of 20.12 ± 0.06, 8.98 ± 0.31, 4.45 ± 0.18, respectively. Among L*, a*, and b* parameters, lightness is the one which was significantly reduced by thermal pasteurization. No significant changes were observed for a*, b*, and BI values of SJ subjected to different processes ($p>0.05$). Since total color difference was actually a function of L*, a*, and b* values, thermal pasteurization also caused significant change in total color difference compared to untreated juice. The total color difference between untreated and UV-C+MH treated samples was not significant.

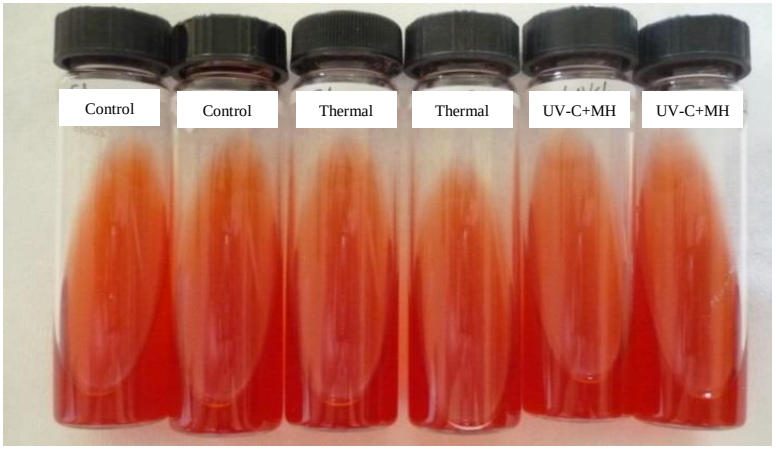


Figure 9.7. The color of strawberry juice collected before and after processing
(Control, Thermal, UV-C+MH refer to untreated, thermal pasteurization at 72 °C, and UV-C assisted with mild heat treatment)

The results of this study indicated that there was no significant differences observed in a*, b*, and BI values of untreated SJ during refrigerated storage. However, lightness decreased significantly which could be attributed to the some enzymes that can cause color loss (Aguiló-Aguayo et al., 2008). Total color difference of untreated SJ, on the other hand, significantly increased with the storage time (Table 9.2). Since color of the final product is a good visual criterion for acceptance of the product by the consumers, any change in the product can affect the preference of the consumers. Besides, undesired color characteristics can be an indicator of inappropriate processing and growth of spoilage microorganisms (Aguilo-Aguayo, Oms-Oliu, Soliva-Fortuny, & Martin-Belloso, 2009). Lightness of untreated strawberry juice was reported as 25 by Bhat and Stamminger, (2015) which is partially in agreement with the current study. However, other color parameters such as a*, and b* reported in the same study were much higher (Bhat & Stamminger, 2015) compared to ones reported in the present study. These differences could be aroused from either characteristics the fruit or color measurement techniques. Cserhalmi et al. (2006) evaluated the total color difference between treated and untreated samples on the basis of varying ranges such as not noticeable (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) (Cserhalmi, Sass-Kiss, Toth-Markus, & Lechner, 2006). Hence, SJ subjected to UV-C+MH showed “not noticeable” total color difference (0.48) (Table 9.2, Figure 9.7). Bhat & Stamminger (2015) investigated the impact of ultraviolet radiation treatments on the physicochemical properties, antioxidants, enzyme activity and microbial load in freshly prepared hand pressed strawberry juice. They

reported a decrease in pH, total soluble solids and titratable acidity, while an increase was observed in the colour parameters (L*, a* and b* values) and clarity of juice (% transmittance). These findings were in line with the results of this study. Additionally, they observed significant increase in the value of ΔE after ultraviolet treatments. This result is comparable to strawberry juice subjected to UV-C+MH heating presented in this study.

9.3.3. Phytochemical Properties of Strawberry Juice after Processing

Total phenolic content (TPC), total anthocyanin content (TAC), and radical scavenging activities of strawberry juice samples was determined before and after each process. TPC of fresh, thermally pasteurized, and UV-C+mild treated samples were compared in Figure 9.8 on the basis of mg gallic acid (GA) equivalent/L. According to variance analysis, no significant differences were observed TPC and RSA properties of untreated, heat treated and UV-C+mild heat treated SJ. This finding was in accordance with some studies previously published in literature. For example, TPC of apple juice was reported to be well retained after UV-C irradiation irrespective of exposure dose (Islam et al., 2016). In another study, no significant differences were reported for TPC and antioxidant activity of white and red grape juices after processing by UV-C irradiation (Pala & Toklucu, 2013b).

On contrary, total anthocyanin content of untreated SJ was significantly reduced by UV-C+mild heat treatment. Thermal pasteurization at 72 °C for 101 s did not significantly alter the TAC of SJ. Bhat and Stamminger (2014) also demonstrated that anthocyanin content of strawberry juice significantly decreased when the juice was subjected to UV-C irradiation for different treatment times up to 60 min (Bhat & Stamminger, 2015). On the other hand, Pala and Toklucu (2011) observed no significant change in monomeric anthocyanin content of pomegranate juice after UV-C irradiation whereas thermal pasteurization caused significant decreasein TAC (Pala & Toklucu, 2011). Depending on the applied UV-C dose, the loss in the content of anthocyanins in red grape juice was reported to be approximately 6-8 % whereas thermal pasteurization caused loss of 11.8 % of anthocyanins (Pala & Toklucu, 2013b). More studies are

required for the phytochemical properties in order to gain a broad knowledge on retention of health related compounds in fruit juices after UV-C irradiation.

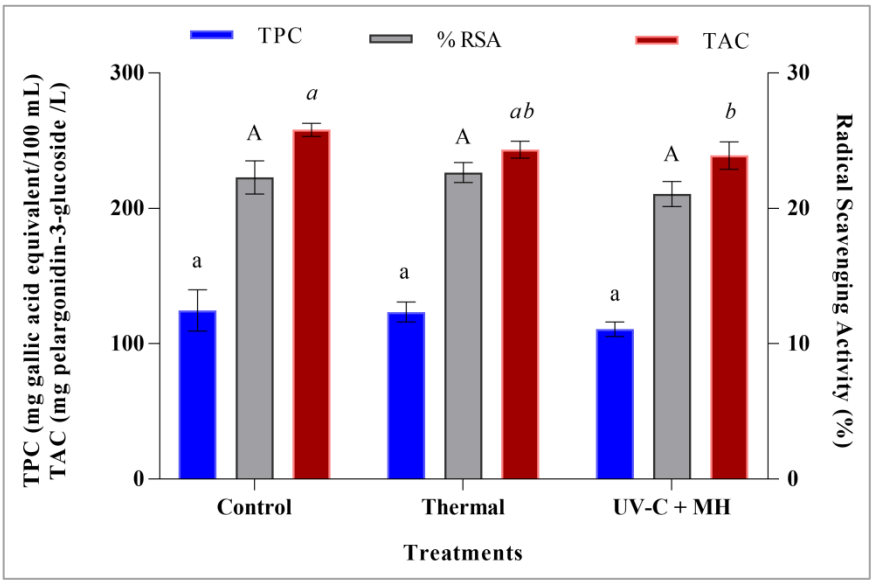


Figure 9.8. Changes in phytochemical properties immediately after processing of strawberry juice

(TPC, RSA, and TAC respectively referred to total phenolic content, radical scavenging activity, total anthocyanin content of strawberry juice subjected to thermal pasteurization at 72 °C and UV-C+mild heat combination. Different letters for the same bar plot indicate significant differences among treatments ($p<0.05$))

9.3.4. Principal Component Analysis

The whole data set including physicochemical and phytochemical properties of SJ after processing at day 0 were evaluated simultaneously by PCA. Figure 9.9 gives score and loading factors as PCA output. From this figure, it could be inferred that the physicochemical and optical parameters were mainly contributed to PC 1 which could explain 68.2 % of the total variance. It is remarkable to indicate that thermal pasteurization was located at a farther region compared to control sample mainly due to the increased total color difference (ΔE) and turbidity after processing. On the other hand, UV-C+MH seemed to have more similar properties to untreated freshly squeezed SJ in terms of titratable acidity, L*, a*, b*, and browning index. PC 2 was able to explain 31.8 % of the total variance. Phytochemical properties, i.e. TPC, TAC, RSA, and absorption coefficient mainly contributed to the PC 2.

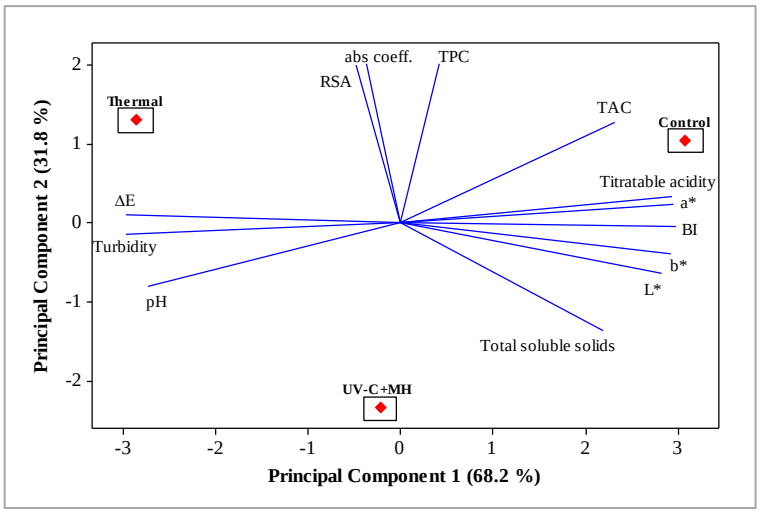


Figure 9.9. PCA output for physicochemical and phytochemical properties of SJ

In the following figure, the data obtained throughout the storage period were evaluated by PCA considering physicochemical and optical properties (Figure 9.10). The numbers used with the abbreviation of treatment refer to the certain day of storage. A clear discrimination of thermal pasteurization and UV-C+MH treatment could be observed in the structure of data according to PC 1 based on physicochemical and optical properties of SJ samples (Figure 9.11). UV-C+MH treated SJ samples showed similar characteristics to freshly squeezed SJ especially after processing at day 0. PC 1 was able to explain 55.4 % of the total variance. PC 2, on the other hand, distinguished the initial and further stages of storage period for each technology by explaining 30.6 % of the total variance in the whole data set.

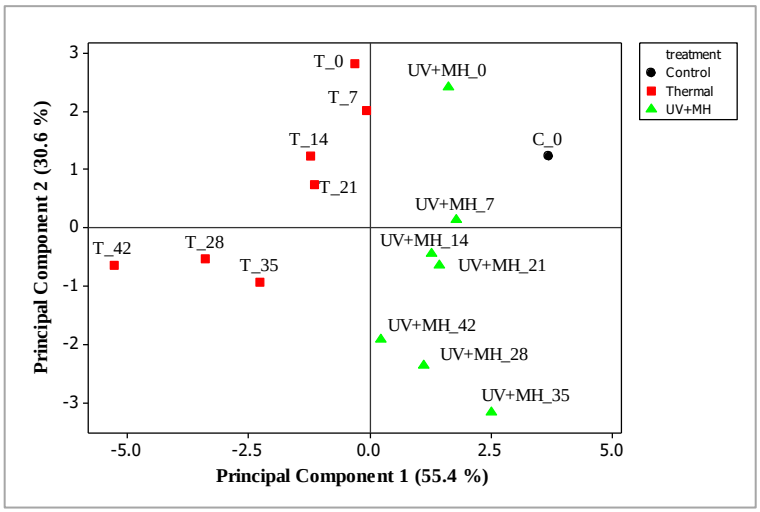


Figure 9.10. Score plot of SJ samples during storage

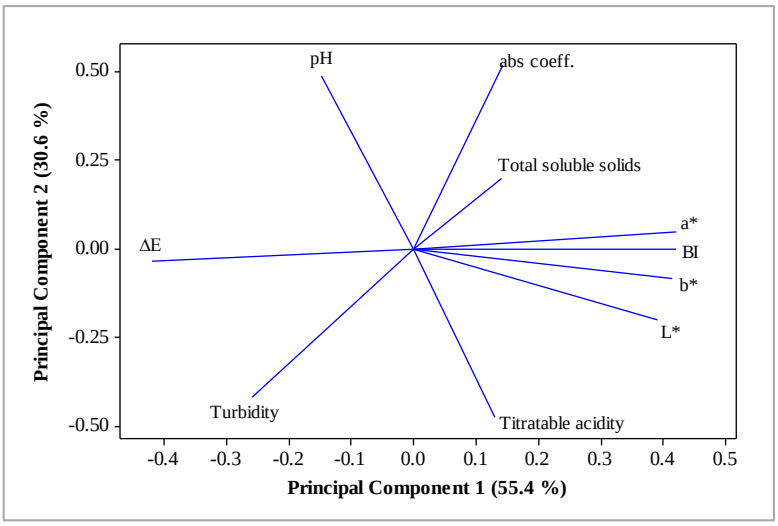


Figure 9.11. Loading plot of SJ samples during storage

9.3.5. Hierarchical Cluster Analysis

Similarities or differences among the strawberry juice samples during storage were visualized by HCA. Figure 9.12 gives the dendrogram as an output of cluster analysis. Since the initial microbial load of untreated SJ was already higher than acceptable limits, the data related to the attributes of freshly squeezed SJ was taken into consideration only for the day 0 of the storage. In accordance with PCA, UV-C+MH treated samples showed more similar properties to freshly squeezed SJ in terms of physicochemical and optical attributes. Therefore, untreated and UV-C+MH treated juices were formed a cluster. With regard to the evaluation of shelf life data, it would worth to indicate that PCA and HCA covered physicochemical and optical properties of SJ. Phytochemical properties were not included to the data matrix since they were only analyzed at day 0. Thus, a different scenario could likely be obtained by the addition of the phytochemical data during storage. Thermally pasteurized SJ samples stored up to 21 days forms another cluster by locating close to untreated juice. These clusters were then followed by UV-C+MH treated SJ stored up to 42 days. Thermally pasteurized strawberry juice samples stored for 28-42 days were formed another cluster at the right side of the dendrogram. Thus, the following cases could be inferred from these clusters:

- UV-C+MH treated SJ showed more fresh-like properties immediately after processing in terms of physicochemical and phytochemical attributes. It should

be noted that although total phenolic content and radical scavenging activities were not significantly affected after UV light treatment, total anthocyanin content reduced significantly after UV-C+MH.

- Then, the similarity to freshly squeezed SJ was followed by the cluster of thermally pasteurized samples stored up to 21st day; UV-C+MH treated samples stored up to 42 days; and finally thermally pasteurized juice stored for 28-42 days.

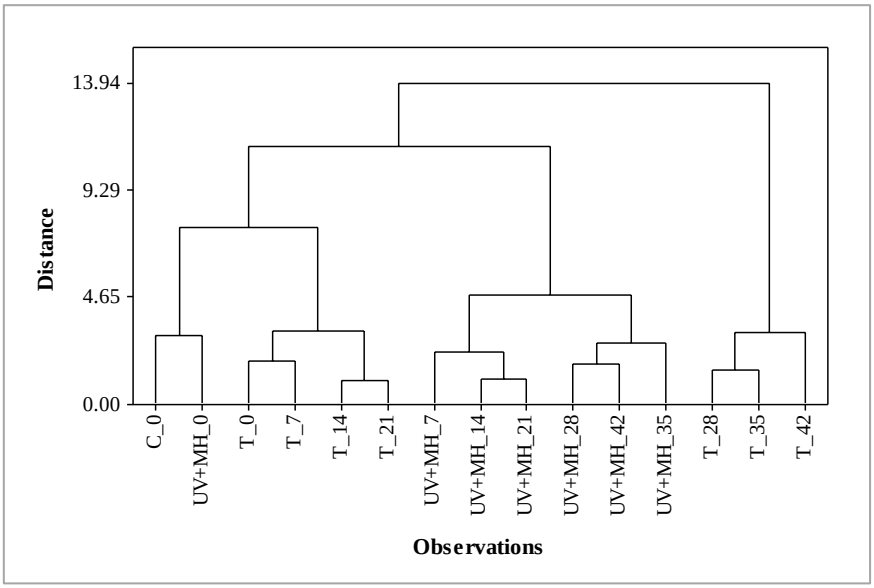


Figure 9.12. Dendrogram for the similarities and differences among treated and untreated strawberry juice during storage

9.4. Conclusions

In this chapter, freshly squeezed SJ was pasteurized either by conventional thermal pasteurization (72 °C for 101 s) or UV-C+MH (four UV-C lamps, UV-C dose of 1.01 J/mL, six times of circulation, exposure time of 58 s per cycle, and flow rate of 3.80 mL/s at 53.9 °C). Microbiological quality and physicochemical properties of SJ were monitored after processing as well as during subsequent refrigerated storage. Additionally phytochemical properties were evaluated after processing. Consequently, the shelf life of SJ was extended up to 42 days by thermal pasteurization and UV-C+MH treatment. In summary, no significant change was observed for pH, total soluble

solids (°Brix), titratable acidity (g/100 mL), and turbidity (NTU) of SJ treated with thermal and UV-C+MH treatments. Thermal pasteurization caused change in total color difference more than UV-C+MH treatment did. Thus, it could be concluded that UV-C irradiation assisted with mild heating can be as effective as moderate thermal pasteurization for the preservation and shelf life extension of strawberry juice. However, total anthocyanin content was negatively affected with the UV-C+MH combination. Principle component analysis and hierarchical cluster analyses showed a clear discrimination among SJ samples based on the physicochemical and optical properties of the control and the UV-C and heat-treated SJ during storage. It could be suggested that UV-C irradiation has a comparable effect on microbial stability at 4 °C and better quality preservation performance than heat treatment. However, special attempts should be considered in order to avoid degradation of anthocyanins.

CHAPTER 10

CONCLUSION

In this Ph.D thesis, the potential of different nonthermal technologies including high pressure processing (HPP), ultrasonication (US), pulsed electrical field (PEF), and UV-C irradiation was explored for mild pasteurization of strawberry juice (SJ). The preservation of quality attributes and retention of bioactive compounds as well as the shelf life of SJ subjected to these nonthermal processes were compared with thermal pasteurization. One of the most important issues for the comparison of characteristics of food products treated by different technologies is the variations or biases that could be originated from less or over processing of the product. In order to overcome this issue, equivalent processing approach was taken into consideration for a fair comparison among processing technologies used in this study. Thereby, equivalent products were obtained on the basis of equivalent degree of microbial inactivation. The pasteurization was redefined by USDA National Advisory Committee on Microbiological Criteria for foods. Therefore "pasteurization" is defined 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." In order to meet this requirement, the juice processors must evaluate their processing operations using a HACCP plan to achieve 5 log reduction in the most resistant (pertinent) microorganism of public health significance. In order to meet FDA requirements, the equivalent inactivation of pertinent microorganism in strawberry juice by selected nonthermal technologies was considered in order to accomplish microbial safety of SJ by mild pasteurization.

In this respect, *E.coli* (ATCC 11775) and *E.coli* K-12 (ATCC 25253) species (i.e. surrogate of *E. coli* O157:H7) adapted to acidic environment were selected as the target microorganism for strawberry juice. *E.coli* (ATCC 11775) was used for HPP, US, and PEF studies conducted at Washington State University. For HPP treatment, inactivation behavior of *E. coli* was monitored at varying pressure and time. Consequently, HPP at 300 MPa for 1 min was able to accomplish at least 5 log reduction in the number of *E. coli* inoculated in SJ. The initial temperature of the

pressurizing liquid was approximately 18 °C and increased during processing by 2.6 °C per 100 MPa due to the compression. Since sonication was not able to achieve desired level of microbial inactivation at ambient temperature, it was combined with mild heating. Accordingly, thermosonication at 55 °C for 3 min revealed 5.69 ± 0.61 log CFU/mL reduction in the number of *E. coli* in SJ under the operating conditions of 5.15 ± 1.34 J/mL acoustic energy, 100 % amplitude (120 μm), continuous pulsing mode. The maximum temperature reached during thermosonication was 56.48 °C. With respect to PEF treatment, a model solution was developed based on citric acid - Na_2HPO_4 buffer solution simulating SJ in terms of pH, titratable acidity, total soluble content, electrical conductivity, viscosity, and specific heat capacity. A central composite design constructed for the inactivation of *E. coli* (ATCC 11775) in MS by PEF was well established the final processing conditions of the treatment. PEF processing achieved 5.53±0.00 log CFU/mL reduction of *E.coli* in model solution considering operating parameters of 35 kV/cm electrical field intensity, 27 μs treatment time, 155 Hz frequency, 350 mL/min flow rate, 2 μs pulse width in monopolar mode. These PEF processing parameters successfully reduced the population of *E. coli* by 5.13±0.15 log CFU/mL in strawberry juice. The maximum temperature achieved at the exit of PEF treatment chamber was 46 °C. Thermal pasteurization at 71.7 °C for 15 s, on the other hand, resulted in complete inactivation of *E. coli* cells in SJ. Thus, processing conditions of each technology were determined on the basis of equivalent degree of *E. coli* inactivation. These processing conditions also achieved remarkable reductions in total mesophilic aerobic and yeast and mold count of fermented SJ with increased natural flora (Chapter 5).

Once the microbial safety was achieved at pre-determined conditions, physicochemical (pH, titratable acidity, total soluble solid (TSS)) and phytochemical (total phenolic content), total anthocyanin content (TAC), and radical scavenging activity (RSA) properties of SJ subjected to these conditions were evaluated as an indicator of overall quality and retention of bioactive compounds of SJ. The pasteurization conditions were determined in Chapter 5 on the basis of equivalent microbial inactivation and validated by directly applying to the freshly squeezed SJ. After processing, the initial load of natural flora of SJ in terms of TMAC and YM was reduced below 2 log CFU/mL by all technologies. According to ANOVA, no significant was observed for the total soluble content and electrical conductivity of SJ samples.

Some significant changes were detected for pH and titratable acidity of SJ samples; however the differences were numerically small. Bioactive constituents of SJ were well retained by nonthermal technologies. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) revealed that HPP and PEF treated SJ samples showed similar properties while thermosonicated and thermally pasteurized SJ samples were similar to each other on the basis of physicochemical properties and bioactive constituents (Chapter 6).

Microbial stability of SJ samples were also monitored in terms of total mesophilic aerobic count (TMAC) and yeast-mold (YM) count during 42 days of refrigerated storage at 4 °C. The microbial shelf life of untreated SJ was extended from 2 weeks to at least 6 weeks by thermal pasteurization, US, and HPP while PEF treatment prolonged the shelf life of SJ up to 5 weeks. Some significant differences were observed in acidity and pH of the treated samples during storage; however, the numerical changes were in small range. No significant difference was observed in the total soluble solid content of the treated juice samples during storage. Multivariate data analysis tools such as PCA and HCA were able to distinguish the SJ samples considering treatments types and storage days. Samples stored up to 14 days were located close to each other while samples from day 21st and forward were close to each other. In general, HPP and PEF treated SJ showed similar properties while thermosonicated and thermally pasteurized samples were similar throughout refrigerated storage (Chapter 7).

E.coli K-12 was used as target microorganism in order to determine the processing conditions for UV-C treatment and thermal pasteurization of strawberry juice studied at Izmir Institute of Technology. Thermal inactivation kinetics of *E. coli* K-12 in strawberry juice were monitored, and consequently thermal pasteurization conditions were determined to be applied at 72 °C for 101 s. On the other hand, different lamp configurations and UV-C intensities were tested on *E. coli* K-12 cells in SJ. UV-C irradiation was not sufficiently enough to meet 5 log reduction requirement of FDA. Thus, UV-C light was combined with mild heating. Accordingly, lethal effect of the treatment increased and 5.78 ± 0.25 log CFU/mL reduction in the population of *E. coli* cells were achieved under the operating conditions of four UV-C lamps, 3.80 ml/s flow rate, 5.8 min treatment time for six cycles, and 1.01 J/mL UV-C dose at 53.9 °C (Chapter 8).

Finally, freshly squeezed strawberry juice was subjected to thermal pasteurization (72 °C for 101 s) and combined UV-C+mild heat treatment under the processing conditions determined in Chapter 8. Both treatments ensured microbial safety, thus no microbial growth was detected during 42 days of refrigerated storage in terms of total mesophilic aerobics and yeast and molds. Neither of the pasteurization methods caused a significant change in pH, titratable acidity, total soluble content, turbidity, absorption coefficient of strawberry juice samples right after processing. No significant change was observed for total phenolic content and antioxidant activities of strawberry juice among treatments, whereas total anthocyanin content of UV-C+mild heat treated strawberry juice was significantly decreased compared to untreated sample (Chapter 9).

Thus, this PhD thesis revealed a fair comparison on quality attributes and retention of bioactive constituents of strawberry juice moderately pasteurized by HPP, US, PEF, and UV-C irradiation. HPP and PEF technologies accomplished a better retention even enhancement of bioactive compounds in SJ immediately after processing. Thermosonication, on the other hand, resulted in similar properties as thermal pasteurization. UV-C irradiation did not show any significant change on total phenolic content and radical scavenging activity; however it significantly reduced total anthocyanin content of SJ compared to untreated juice. Among all processes, HPP and PEF can be favorably reasonable treatments to extend the microbial shelf life and to retain the bioactive compounds of strawberry juice. Enzyme inactivation in strawberry juice by these technologies can be suggested as a future study.

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APPENDIX A

NUTRITIONAL CHARACTERISTICS OF STRAWBERRY

Table A.1. Nutrient profile of strawberry fruit (Source: Giampieri et al. (2012))

Type	Nutrient	Per 100 g
Proximates	Water (g)	90.95
	Energy (kcal)	32
	Protein (g)	0.67
	Ash (g)	0.40
	Total lipid (g)	0.30
	Carbohydrate (g)	7.68
	Dietary fiber (g)	2.0
	Sugars (g)	4.89
	Sucrose (g)	0.47
	Glucose (g)	1.99
	Fructose (g)	2.44
Minerals	Calcium (mg)	16
	Iron (mg)	0.41
	Magnesium (mg)	13
	Phosphorus (mg)	24
	Potassium (mg)	153
	Sodium (mg)	1
	Zinc (mg)	0.14
	Copper (mg)	0.048
	Manganese (mg)	0.386
	Selenium (mg)	0.4
Vitamins	Vitamin C (mg)	58.8
	Thiamin (mg)	0.024
	Riboflavin (mg)	0.022
	Niacin (mg)	0.386
	Pantothenic acid (mg)	0.125
	Vitamin B6 (mg)	0.047
	Folate (mg)	24
	Choline (mg)	5.7
	Betaine (mg)	0.2
	Vitamin B12 (mg)	0
	Vitamin A, RAE (mg)	1
	Lutein + zeaxanthin (mg)	26
	Vitamin E, α-tocopherol (mg)	0.29
	β-tocopherol (mg)	0.01
	γ-tocopherol (mg)	0.08
	δ-tocopherol (mg)	0.01
	Vitamin K, phylloquinone (mg)	2.2

Table A.2. Flavonoids in strawberry (Source: Giampieri, et al. 2012)

Group	Compound	References
Anthocyanins	Cyanidin-3-glucoside	da Silva et al., 2007
	Cyanidin-3-rutinoside	da Silva et al., 2007
	Cyanidin-3-malonylglucoside	da Silva et al., 2007
	Cyanidin-3-malonylglucosyl-5-glucoside	da Silva et al., 2007
	Pelargonidin-3-galactoside	da Silva et al., 2007
	Pelargonidin-3-glucoside	da Silva et al., 2007
	Pelargonidin-3-rutinoside	da Silva et al., 2007
	Pelargonidin-3-arabinoside	da Silva et al., 2007
	Pelargonidin-3,5-diglucoside	da Silva et al., 2007
	Pelargonidin-3-malylglucoside	da Silva et al., 2007
	Pelargonidin-3-malonylglucoside	da Silva et al., 2007
	Pelargonidin-3-acetylglucoside	da Silva et al., 2007
	5-pyranopelargonidin-3-glucoside	Aaby et al., (2007)
Flavonols	Quercetin-3-glucuronide	Aaby et al., (2007), Wang & Miller, (2009)
	Quercetin-3-malonyglucoside	Aaby et al., (2007)
	Quercetin-rutinoside	Aaby et al., (2007)
	Quercetin-glucoside	Wang & Miller, (2009)
	Quercetin-glucuronide	Aaby et al., (2007)
	Kaempferol-3-glucoside	Aaby et al., (2007), Wang & Miller, (2009)
	Kaempferol-3-malonyglucoside	Aaby et al., (2007)
	Kaempferol-coumaroyl-glucoside	Aaby et al., (2007)
	Kaempferol-glucunoride	Wang & Miller, (2009)
Flavanols	Proanthocyanidin B1 (EC-4,8-C)	Aaby et al., (2007)
	Proanthocyanidin trimer (EC-4,8-EC-4,8-C)	Aaby et al., (2007)
	Proanthocyanidin B3 (C-4,8-C)	Aaby et al., (2007)
	(β)-catechin	Aaby et al., (2007)

APPENDIX B

CALIBRATION CURVES FOR TOTAL PHENOLIC CONTENT ANALYSIS

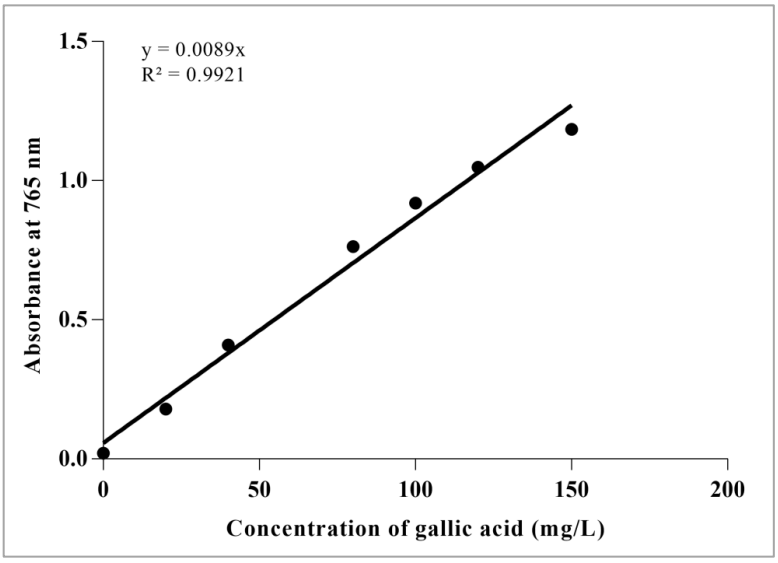

Figure B.1. Calibration curve for total phenolic content analysis conducted at WSU

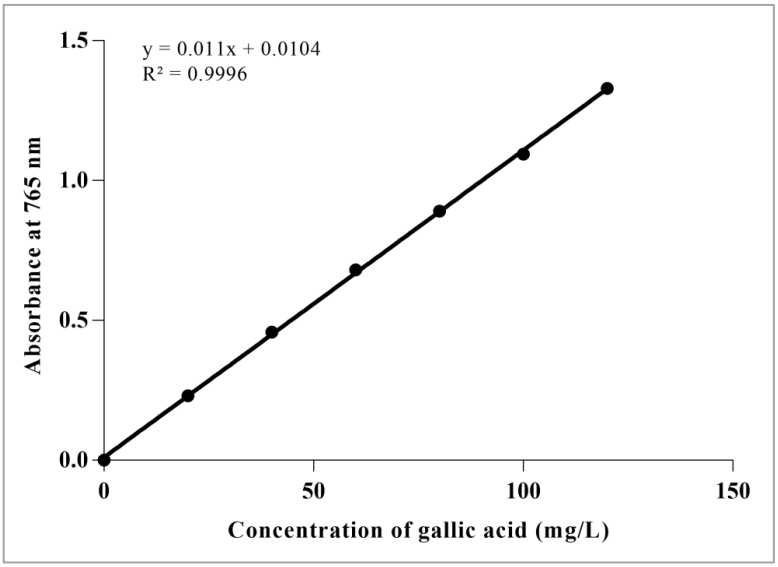

Figure B.2. Calibration curve for total phenolic content analysis conducted at IZTECH

APPENDIX C

MATERIALS USED FOR THIS PHD THESIS

The details of the materials used for the chemical and microbiological analyses of this PhD thesis were listed below.

For Chapter 5-7 (Studies conducted at WSU):

Citric acid	J.T. Baker 0110-01, 500 g
Citric acid monohydrate	Sigma C-7129, 500 g, CAS=5949-29-1
D-(-)-Fructose	Sigma F3510, 5 KG, CAS=57-48-7
D-(+)-Glucose	Sigma G7021, 5kg, CAS=50-99-7
DPPH (2,2-Diphenyl-1-picrylhydrazyl)	Sigma D9132, 1 g, CAS=1898-66-4
Folin-Ciocalteu's phenol reagent	Sigma F9252, 500 mL, 2 N.
Gallic acid	Acros Organics 410862500, 98 %, CAS=149-91-7
Hydrochloric acid (HCl)	J.T. Baker 9535-01, ACS 36.5-38 %, CAS=7647-01-0
Methanol	Fisher Scientific A412-4, 4 L, Certificate ACS reagent grade, CAS=67-56-1
Phosphoric acid (H_3PO_4)	J.T. Baker 0260-05, ACS, CAS=7664-38-2
Potassium chloride (KCl)	J.T. Baker 3040-01, CAS= 7447-40-7
Sodium acetate trihydrate, crystal	J.T. Baker 3460-01
Sodium carbonate (Na_2CO_3)	Sigma S2127, 500 g, CAS=497-19-8
Sodium chloride (NaCl)	Fisher Scientific S271-3, 3 KG, CAS=7647-14-5
Sodium hydroxide (NaOH)	J.T. Baker 3722-5, CAS=1310-73-2
Sodium phosphate dibasic anhydrous	J.T. Baker 3828-01, ACS reagent, CAS=7558-79-4
Trolox	Sigma 238813, 1 g, CAS=53188-07-1 ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid)
EMB Agar	Fisher Scientific R453402
Enterococcosel Agar	BD, BBL, 212205
MacConkey	BD, Difco 212123
Nutrient Broth	BD, Difco 234000
Oxford Agar	Sigma 75805, 500 g
Pepton	BD, Bacto 211677
Plate Count Agar (PCA)	BD, Difco 247940
Potato Dextrose Agar (PDA)	BD, Difco 213400

Tryptic Soy Agar (TSA)	BD, Difco, Soybean-Casein Digest Agar Medium 236950
Tryptic Soy Broth (TSB)	BD, Bacto 211825
Violet Red Bile Agar (VRBA)	BD, Difco 211695
Yeast Extract	BD, Bacto 212750

For Chapter 8 & Chapter 9 (Studies conducted at IZTECH):

Antibiotic	
Borax (disodium tetraborate decahydrate)	Merck 106303, 1 kg
DPPH	
Folin-Ciocalteu Reagent	Merck 109001, 100 mL
Gallic acid	Merck 842649, 25 g
Methanol	
Nutrient Broth	Merck 105443, 500 g
Pepton water buffered	Merck 107228, 500 g
Plate Count Agar	Merck 105463, 500 g
Potassium chloride (KCl)	Merck 104936, 1 kg
Potassium iodate	Merck 105051, 100 g
Potassium iodide	Merck 105043, 250 g
Potato dextrose Agar	BD DIFCO 213400, 500 g
Sodium acetate	
Sodium carbonate	Merck 106392, 1 kg
Sodium chloride (NaCl)	Merck 106404, 1kg
Sodium hydroxide (NaOH)	Merck 106498, 1 kg
Tartaric acid	
Trolox	
Tryptic Soy Agar	Merck 105458, 500 g
Tryptic Soy Broth	Merck 105459, 500 g
Violet Red Bile Agar	Merck 101406, 500 g

VITA

Semanur Yildiz was born in Kars, Turkey in 1988. She received Bachelor degree in Food Engineering from Uludag University, Bursa, in 2010. She remained at the same university for Master of Science degree in Food Engineering completed in June 2012. She later worked as a research assistant at Department of Food Engineering of Sakarya University for 3 months. In September 2012, she has started her education at İzmir Institute of Technology (IZTECH) in order to get the degree of Doctor of Philosophy in Food Engineering. She has been working as a research assistant at IZTECH since December 2012. During her MSc and PhD periods, she has gained experience in the field of food science, biotechnology and food processing technologies as a visiting scholar in Germany (University of Bonn, Rheinische Friedrich-Wilhelms-Universität Bonn), Mexico (Tecnológico de Monterrey) and United States (Washington State University). Semanur has become student representative of Asia at Nonthermal Processing Division (NPD) of Institute of Food Technologists (IFT) for the period of March 2016 – June 2017. Currently, she has been working on the application of different nonthermal processing technologies on fruit juices.