

Article

Effect of Stand-Alone and Combined Ultraviolet and Ultrasound Treatments on Physicochemical and Microbial Characteristics of Pomegranate Juice

Tareq A. M. Alabdali ¹, Necattin Cihat Icyer ², Gulsum Ucak Ozkaya ^{1,3} and Muhammed Zeki Durak ^{1,*}

¹ Department of Food Engineering, Faculty of Chemical & Metallurgical Engineering, Yildiz Technical University, 34210 Istanbul, Turkey; tam1985a@gmail.com (T.A.M.A.); gucak@yildiz.edu.tr (G.U.O.)

² Department of Food Engineering, Faculty of Engineering and Architecture, Mus Alparslan University, 49100 Mus, Turkey; n.icyer@alparslan.edu.tr

³ Department of Food Engineering, Faculty of Engineering and Architecture, Bitlis Eren University, 13000 Bitlis, Turkey

* Correspondence: mzdurak@yildiz.edu.tr; Tel.: +90-(212)-383-4578; Fax: +90-(212)-383-4571

Received: 7 July 2020; Accepted: 24 July 2020; Published: 7 August 2020



Featured Application: This paper features the design of modern pasteurization methods based on two combined non-thermal pasteurization methods. This combined method can be used as an alternative to the conventional thermal pasteurization method as it protects the nutrient content better.

Abstract: The objective of this study was to determine the combined usage possibilities of ultraviolet (UV) and ultrasonic (US) processes in the pasteurization of pomegranate juice. For this purpose, UV, US, and combined UV+US pasteurization of pomegranate juice were optimized using experimental designs, such as the Central Composite Design (CCD) and Factorial Design (FD), and compared with the conventional pasteurization process. Total phenolic content (TPC), color a*, water-soluble dry matter (°Brix), turbidity, anthocyanin, DPPH, HPLC TPC, and yeast and mold count were used as quality parameters during all of the processes. The results showed that the application of 50 °C, 3.5 L/min flow rate and 5.1 mW/cm² UV dose, and 10 min US (200 Watt) together reduced the microbial population below the detection limits. The integration of UV+US processes into the pasteurization process could limit microbial activity at lower temperatures and times than the conventional pasteurization process, thus preserving the existing bioactive compounds.

Keywords: pasteurization; pomegranate juice; ultrasound; ultraviolet; optimization; experimental design

1. Introduction

Consumers have had an increasing interest in fruit juices which includes bioactive compounds that provide positive health outcomes. These bioactive compounds include polyphenolic compounds with anticarcinogenic, antihypertensive, antimicrobial, and antioxidant properties [1,2]. Pomegranate (*Punica granatum* L.) which is a widespread fruit used in sauces and jams is also used as a supplemental material worldwide. This is one of the scarce fruits with significant functional characteristics, and its manufacturing rates are increasing, as well. There is a lot of interest in pomegranate fruit juice (PJ) due to its high phenolic compound content, for example, anthocyanins, ellagic tannins, and catechins. In addition to the many health benefits of bioactive compounds, anthocyanins are responsible for the bright red color, which is one of the essential properties of PJ. This impacts one of the main concerns of consumers [3–5].

A serious concern with PJ is microbial contamination with acid-resistant yeasts or mold, which lead to deterioration of nutritional and sensory properties such as color, smell, and flavor [6]. The percentage of microbial contamination depends on the process of manufacturing PJ, fruit type, harvest time, and processing conditions [7].

The expectation of consumers for healthy food is increasing with the improvement of technology. Nowadays, to increase the shelf life of food, microorganisms and enzymes are inhibited by chemical, thermal, or combined processes [8]. The conventional methods for thermal pasteurization use the conditions that are 63 °C–30 min, 72 °C–15 s, or 90 °C–5 s [9]. Conventional thermal pasteurization inhibits microorganisms that cause damage to the juices and raises the shelf life. This method still has disadvantages because it has negative effects on the sensory properties and especially on functional compounds [10]. In particular, the heat treatments affect the content of anthocyanins and the color of the juices. Anthocyanins decompose under the influence of heat treatment, causing undesirable color changes to brown or colorless. Therefore, alternative methods are needed to maintain the color of fruit juices [5]. Although food processing methods provide fresh, safe, and rich foods, the demand for alternative methods that minimize the effects on nutrient content is increasing [11]. Some of the non-thermal methods with the potential to replace thermal processing of foods include ultrasonic and ultraviolet treatments [12,13].

Ultraviolet radiation (UV) is among the non-thermal process technologies used to sterilize and preserve food and juices. The wavelength is between 200–280 nm, and this feature has a definite effect on microorganisms like yeast and mold, and bacteria [14]. UV lights are largely adsorbed by microorganism DNA and block DNA transcription and bond translation in the same DNA strand via adjacent pyrimidine bases [15].

The National Advisory Board on Microbiological Criteria for Food Department of Agriculture of the USA stated that preservation by non-thermal technologies, including ultraviolet light, provide scientific parameters for the pasteurization of juices. This was a 5 log reduction of the most resistant microorganism of threatening to public health [16,17]. UV radiation was proven to be a safe method used in the pasteurization of juices by the US Food and Drug Administration (FDA) [18] and The United States Department of Agriculture (USDA). The effectiveness of UV light in apple, grape, cranberry, and grape juice and orange juice has also been demonstrated by various studies [15,19].

Ultrasound processing (US) is one of the non-thermal techniques used to preserve foods where the thermal coefficients affect the nutritional value of food products like fruit juices. Ultrasound processes have shown that they have a positive effect on food manufacturing in recent years. They have also been reported to inhibit microorganisms and enzymes [20–22]. Ultrasonic techniques make sound waves (commonly at 20 kHz) that vibrate with frequencies above the human audible upper limit [23]. The mechanism of ultrasound power produces strong ripples from cavitation in liquid solutions according to the juice characteristics, air presence, and ultrasonic system acoustic power. US induces cavitation by forming microscopic gas bubbles in a liquid. When these bubbles explode, they produce intense shock waves and free radicals across the cell membrane, which contributes to microbial inactivation [24,25]. There are studies on the use of ultrasound to inhibit microorganisms and enzymes in orange juice [26] and red grape juice [27]. Upon used ultrasound in food technology, it is able to be improved the reaction conditions by lowering temperatures and reducing the processing time with less external input [28].

In the present study, we aimed to optimize various parameters in PJ pasteurization with a face-centered, Central Composite Design (CCD) and a Factorial Design (FD). Comparisons of optimal parameters among UV, US, UV+US, and conventional pasteurization treatments were also presented. The most crucial aim of this study is to optimize UV and US methods and to offer an alternative method to the conventional method by obtaining a method combined with the optimum conditions obtained from experimental design results.

2. Materials and Methods

2.1. Materials

Fresh pomegranate fruits were supplied from a local market in Istanbul, Turkey. The fruits were selected considering their freshness, color, size and absence of microbial or mechanical damage. All chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of Pomegranate Juice

Firstly, pomegranate fruits were washed using tap water. The seeds were separated manually and fruit was then squeezed in a juicer (King P-1120 Vitamix Juicer 400W, Istanbul, Turkey). The juices were freshly squeezed before each pasteurization method.

2.2.2. Conventional Pasteurization Treatment

In the conventional pasteurization process, the temperature was set at 72 °C in the water bath (Daihan, WUC-D10H, Seoul, Korea) and the juice was allowed to be pasteurized at this temperature for 15 sec. Conventionally pasteurized PJ was used as a control sample to compare methods.

2.2.3. Ultraviolet Treatment

PJ samples were pasteurized with a UV pasteurization device (CiderSure 3500, FPE Inc, Rochester, MN, USA) under different process conditions (temperatures, flow rates, UV doses) determined by the experimental design. UV exposed PJ samples were aseptically filled into sterile glass bottles (100 mL) and covered immediately for further experiments. Optimum conditions for UV treatment were determined considering TPC and yeast and mold count.

2.2.4. Ultrasound Treatment

PJ with the amount of 250 mL was put into a vessel glass with cooling cylindrical jacket and it was exposed to ultrasonic liquid processing (Hielscher UIP1000, 1000 W–20 kHz, Teltow, Germany) with a 22 mm diameter probe (Hielscher sonotrode BS4D22, Teltow, Germany) and a cell movement (Hielscher, FC100L1K–1S, Teltow, Germany). The temperature of the cooling water was 25 °C, and the flow of water was 2.1 L/min. Considering the experimental design, ultrasound power (50%, 75%, and 100%; 165, 200, 295 W, respectively) was applied to the PJ. The temperature was adjusted to 40, 50, and 60 °C. The increase in the power of sonication resulted in an increase in the temperature of the sample, as well. Since it was difficult to decrease/increase the temperature using 50% (165 W), 75% (200 W), and 100% (295 W) ultrasound power, the temperature of the sample was kept constant at the desired temperature as using a cooling circulator (Daihan WCRP22, Seoul, Korea). Using 165, 200, and 295 W power, the sample temperatures were set at 40 °C, 50 °C, and 60 °C, respectively. Optimum conditions for US treatment were decided considering TPC and yeast and mold count.

2.2.5. UV and Ultrasound Treatment

After determining optimum conditions for UV and US processes, these two methods were used in combination. Briefly, PJ was first pasteurized in UV using predetermined optimum conditions and then immediately submitted to the ultrasound device, according to the optimum US design. The final PJ was used for further analysis.

The UV, US, and UV+US treated samples were immediately conducted the physicochemical analysis, the analysis for bioactive properties, phenolic profile by HPLC, and microbiological analysis. When all analyzes cannot be performed in one day, treated PJ was stored at +4 °C. All samples were stored at −20 °C after all analyzes were completed.

2.2.6. Physicochemical Analysis

Color a^* values, which are indicative of redness, were determined by colorimeter (Konica Minolta CR-400, Tokyo, Japan). °Brix values of the PJ were determined using the Abbe refractometer at 20 °C. For determination of the turbidity value of PJ, 7 mL of a sample was combined with 1 mL of sterilized water and mixed gently to ensure liquid mixing. The mixture was then measured by a spectrophotometer at 660 nm.

2.2.7. Bioactive Properties

Total phenolic content (TPC) was determined in the way mentioned by Singleton, Orthofer [29]. Namely, 0.5 mL of the sample was incorporated 2.5 mL of Folin–Ciocalteu’s solution (0.2 N) and 2 mL of Na_2CO_3 (7.5% *w/v*) were added to the mixture. It was then mixed for 1 min and left for a half-hour at room temperature in a dark condition. Following this, the absorbance values of the samples were measured at 760 nm wavelength using a spectrophotometer device (Shimadzu UV-1800, Kyoto, Japan). Results were expressed as milligrams of gallic acid equivalent per 1000 mL of sample weight (mg GAE/1000 mL extract).

The anthocyanin concentration was determined by considering the absorbance values measured at different pH values [30]. The absorption of the extracts was measured at 520 and 700 nm. The anthocyanin content of samples was calculated according to the following equation:

$$\text{Anthocyanin content} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{A \times MW \times DF \times 1000}{\epsilon \times 1}.$$

The results were expressed as cyanidin-3-glucoside equivalents for pomegranate juice using the absorption value (A) of samples, the molecular weight (MW) of 449.2 g/mol, and an absorptive coefficient (ϵ) of 26.900 L/mol and with a dilution factor (DF) of samples.

The antiradical activity was determined by a method described by Durak and Ucak [31]. This method determines the free radical scavenging activity of pomegranate juice. The 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical was used to test the antiradical activity of PJ. The DPPH solution (0.1 mM) was prepared in methanol. A 0.1 mL sample solution was added to each tube, and a 3.9 mL DPPH solution was incorporated and mixed with the vortex mixer for 1 min. The samples were incubated for 30 min in a dark environment, and the absorbance was measured at 520 nm. The antiradical activity (AA%) value was calculated by the following equations:

$$(AA\%) = (A_{\text{control}} - A_{\text{sample}}) \times 100$$

where A_{control} and A_{sample} were the absorbance values of the solution with and without the presence of the samples, respectively.

2.2.8. Phenolic Profile by HPLC

The phenolic profile in PJ was determined by High-Performance Liquid Chromatography (HPLC). Curves were prepared for standard calibration by employing gallic acid, *p*-hydroxybenzoic acid, caffeic acid, protocatechuic acid, catechin, *o*-coumaric acid, syringic acid, *p*-coumaric acid, *m*-coumaric acid, ferulic acid, myricetin, quercetin, kaempferol, and chrysin.

The samples were filtered through a membrane filter of 0.45 μm . They were then evaluated in a system with a Shimadzu HPLC (SPDM20A DAD detector, LC-20AD pump, SIL-20A HT autosampler, DGU-20A5R degasser, CTO-10ASVP column oven, and CMB-20A communications bus module, Shimadzu Corp., Kyoto, Japan). Separations were performed at 40 °C on an Intersil ODS C-18 reversed-phase column (250 mm $\times$ 4.6 mm length, 5 μm particle size). The mobile phase contained solution A (distilled water with 0.1% (*v/v*) acetic acid), and solution B (acetonitrile with 0.1% (*v/v*) acetic acid). Gradient elution was conducted as follows: 10% B (0–2 min), 10–30% B (2–27 min), 30–90% B (27–50 min), and 90–100% B (51–60 min), and at 63 min it was returned to initial conditions.

The flow rate was 1 mL/min. Chromatograms were observed at 278, 320, and 360 nm. Identification and quantitative analyses were performed based on the retention times and the external norm curve. HPLC–DAD results were given as µg/g PJ.

2.2.9. Microbiological Analysis

Microbial analyzes were performed immediately to determine the effectiveness of the UV, US, and UV+US combined methods since the main purpose of pasteurization is to inhibit bacteria or yeast and mold that cause spoilage. Analysis for yeast and mold count was performed on the same day of UV, US, and UV+US treatment. The yeast and mold count was conducted on DRBC agar using the spread plate technique. Following incubation at 25 °C for five days, the results were expressed log CFU/mL.

2.2.10. Experimental Design and Statistical Analysis

Experimental design and 3D graphics were generated using the Design-Expert program version 7.0.0 (State-Ease Inc., Minneapolis, MN, USA). One-way analysis of variance (ANOVA) and Duncan analysis were performed using the Windows statistical program SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Three independent variables were used to study the effects of (temperature, flow rate, and UV dose) in the UV pasteurization. Similarly, the effects of three independent variables were studied using a face-centered CCD response surface methodology (temperatures, ultrasound power on/off, and times) in the US pasteurization.

3. Results

The yeast-mold count, TPC, °Brix, and color a* results of the pomegranate juice sample that had not been subjected to any treatment are shown in Table 1.

Table 1. Result for total phenolic content (TPC), turbidity, °Brix, color a*, and yeast and mold count of unprocessed pomegranate juice.

TPC (mg GAE/L)	Turbidity	°Brix Value	Color a*	Yeast and Mold Count log CFU/mL
1078 ± 5.72	4.2 ± 0.19	14 ± 0.0	2.3 ± 0.0	5.3 ± 0.1

The values are expressed as mean ± standard deviation (replications = 3).

3.1. Optimization of Parameters and Design Checks in UV Processing

The responses obtained from 12 samples with UV pasteurization according to the experimental design are given in Table 2. The results showed that the TPC amounts, turbidity values, °Brix values, color a* values, and yeast and mold count at the experimental points were ranged from 840 to 1060 mg GAE/L, 3.59 to 4.61 NTU, 14–16 °Bx, 2.3 to 2.5, and <0.5 to 3.92 log CFU/mL, respectively.

ANOVA analysis of the data obtained from the experimental design and the interaction of the factors with the responses are given in Table 3. The results showed that temperature and flow rate parameters were statistically significant for the amount of yeast and mold, and the UV dose was not statistically important. Considering the results, the increase of the UV dose would decrease the microbial growth at 40 °C and 50 °C temperature applications. The microorganism growth is under the detection limit because of the simultaneous UV application at 60 °C. Since the microbial growth was already undetectable at this temperature, the intensity of the UV dose applied was statistically insignificant. UV treatment resulted in an average of 1–2 log reduction of the yeast and mold count. The value of R² was 0.999, showing a strong linkage between the independent parameters and the responses.

Table 2. Experimental parameters in the UV process and the observed response values for pomegranate juice.

Run Order	X ₁	X ₂	X ₃	Phenolic Content (mg GAE/L)	Turbidity	°Brix	Color a*	Yeast and Mold Count log CFU/mL
1	50	1.5	1 LAMP	920 ± 4.42 ^g	4.01 ± 0.007 ^j	14 ± 0.0 ^c	2.3 ± 0.08 ^{ab}	3.09 ± 0.96 ^g
2	60	1.5	1 LAMP	857 ± 0.44 ⁱ	3.59 ± 0.000 ^k	15 ± 0.0 ^b	2.3 ± 0.07 ^{ab}	<0.5 ^k
3	50	3.5	2 LAMPS	969 ± 0.59 ^d	3.99 ± 0.007 ^g	15 ± 0.0 ^b	2.3 ± 0.05 ^{ab}	3.11 ± 0.00 ^f
4	40	1.5	1 LAMP	1055 ± 2.21 ^{ab}	4.58 ± 0.007 ^b	14 ± 0.0 ^c	2.3 ± 0.09 ^{ab}	3.75 ± 0.82 ^c
5	40	3.5	2 LAMPS	1053 ± 0.00 ^b	4.34 ± 0.000 ^d	14 ± 0.0 ^c	2.3 ± 0.03 ^{ab}	3.86 ± 0.50 ^b
6	50	3.5	1 LAMP	1020 ± 0.00 ^c	4.05 ± 0.003 ^f	15 ± 0.0 ^b	2.3 ± 0.00 ^{ab}	3.34 ± 0.50 ^e
7	50	1.5	2 LAMPS	890 ± 0.14 ^h	4.19 ± 0.004 ^e	15 ± 0.0 ^b	2.3 ± 0.05 ^{ab}	2.97 ± 0.58 ^h
8	60	1.5	2 LAMPS	840 ± 0.25 ^j	3.61 ± 0.014 ^k	16 ± 0.0 ^a	2.5 ± 0.03 ^{ab}	<0.5 ^k
9	40	3.5	1 LAMP	1060 ± 0.00 ^a	4.41 ± 0.000 ^c	14 ± 0.0 ^c	2.3 ± 0.06 ^{ab}	3.92 ± 0.56 ^a
10	40	1.5	2 LAMPS	938 ± 1.80 ^f	4.61 ± 0.021 ^a	14 ± 0.0 ^c	2.3 ± 0.01 ^{ab}	3.59 ± 0.50 ^d
11	60	3.5	1 LAMP	958 ± 0.00 ^e	3.82 ± 0.004 ^h	15 ± 0.0 ^b	2.3 ± 0.00 ^{ab}	2.00 ± 0.00 ⁱ
12	60	3.5	2 LAMPS	925 ± 0.00 ^g	3.76 ± 0.014 ⁱ	15 ± 0.0 ^b	2.5 ± 0.07 ^a	1.69 ± 0.82 ^j

X₁ (Temperature, °C), X₂ (Flow rate, L/min), X₃ (UV Lamp 1 and 2 doses are 5.1 and 10.1 mW/cm², respectively).
 a–k: Mean with superscripts of different small letters in the same columns are significantly different as Duncan test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3).

Table 3. ANOVA analysis results of Factorial Design (FD) for yeast and mold count in UV pasteurization process (log CFU/mL).

Source	Sum of Squares	Mean of Square	F Value	p-Value
Model	21.50	2.39	227.37	0.0044
X ₁	17.94	8.97	853.49	0.0012
X ₂	1.70	1.70	162.02	0.0061
X ₃	0.06	0.06	6.14	0.1315
X ₁ X ₂	1.79	0.89	85.07	0.0116
X ₁ X ₃	0.00	0.00	0.11	0.9046
X ₂ X ₃	0.01	0.01	0.81	0.4626
Residual	0.02	0.01		
Correction Total	21.52			
R ² = 0.9990				

X₁ (Temperature, °C), X₂ (Flow rate, L/min), X₃ (UV Lamp doses), $p < 0.05$ indicates statistical significance, ANOVA refers to the analysis of yeast and mold count (log CFU/mL).

Temperature, flow rate, and UV dose parameters were not statistically significant ($p > 0.05$) when the TPC amount of samples treated with UV was considered. Even though the effect of the temperature, flow rate, and UV dose on TPC values in the UV pasteurization process was not statistically significant, the TPC amount of samples decreased. Phenolic compounds are adversely affected and reduced at higher temperatures and UV doses. The °Brix value increased due to the increase in temperature, which is statistically significant ($p < 0.05$) while the effects of other factors on the °Brix value of samples are statistically insignificant ($p > 0.05$). As for the ANOVA test results for turbidity values, only the temperature is statistically significant for turbidity ($p < 0.05$). The color a* value of samples was not statistically affected by temperature, UV dose, and flow rate ($p > 0.05$).

Optimization tests were performed for yeast and mold count and TPC amount responses in the design. UV applications with the highest temperature (60 °C), the lowest flow rate (1.5 L/min), and used either 1 lamp or 2 lamps inhibited almost all yeast and mold growth. Even if these conditions were seen as the best condition, the optimum condition was obtained evaluating together with the TPC and yeast and mold count by the design expert program.

For the optimization process, although the amount of TPC tended to increase–decrease depending on three factors, it was not statistically affected by low temperature and short processing time.

In addition, turbidity, °Brix, and color a^* values were not statistically significant enough to represent the whole experimental design. Therefore, the optimization process was carried out considering the conditions where the yeast and mold content is minimum, and the TPC amount is maximum. Considering these conditions, the optimum condition for stand-alone UV treatment was determined as 50 °C, 1.5 L/min, and 2 lamps by the design expert program. Desirability, which is a criterion for accuracy in optimization processes, was found to be 1.000.

3.2. Optimization the Parameters and Verification of Design in US Processing

The responses obtained from 18 experimental points with US pasteurization according to the CCD are shown in Table 4. For 18 experimental points, the values of TPC, turbidity, °Brix, color a^* , and yeast and mold count ranged from 1079 to 1481 mg GAE/L, 3.88 to 4.71 NTU, 14 to 15 °Bx, 3.03 to 3.88, and <0.5 to 4.3 log CFU/mL, respectively.

Table 4. Experimental parameters in the ultrasonic (US) treatment and the observed response values for pomegranate juice.

Run Order	X ₁	X ₂	X ₃	Phenolic Content (mg GAE/L)	Turbidity	°Brix	Color a^*	Yeast and Mold Count log CFU/mL
1	50	10	ON	1252 ± 1.81 ^d	4.23 ± 0.000 ^{bcde}	15 ± 0.0 ^a	3.46 ± 0.00 ^f	3.4 ± 0.67 ⁱ
2	40	10	OFF	1103 ± 0.27 ^{gh}	4.11 ± 0.003 ^h	15 ± 0.0 ^a	3.46 ± 0.01 ^f	4.4 ± 0.80 ^a
3	50	15	ON	1273 ± 0.18 ^d	4.24 ± 0.011 ^{bcd}	15 ± 0.0 ^a	3.46 ± 0.00 ^f	3.0 ± 0.96 ^k
4	40	15	ON	1138 ± 0.18 ^f	4.20 ± 0.003 ^{def}	15 ± 0.0 ^a	3.54 ± 0.02 ^c	4.0 ± 0.5 ^e
5	60	5	ON	1310 ± 0.18 ^c	4.28 ± 0.018 ^b	15 ± 0.0 ^a	3.53 ± 0.05 ^d	3.1 ± 0.38 ^j
6	60	10	ON	1358 ± 0.18 ^b	4.28 ± 0.018 ^b	15 ± 0.0 ^a	3.16 ± 0.00 ^h	2.8 ± 0.20 ^l
7	60	5	OFF	1173 ± 0.18 ^e	4.25 ± 0.004 ^{bcd}	14 ± 0.0 ^b	3.03 ± 0.03 ^j	3.9 ± 0.79 ^f
8	50	5	OFF	1116 ± 0.02 ^{fg}	4.14 ± 0.003 ^{fgh}	14 ± 0.0 ^b	3.53 ± 0.00 ^d	4.1 ± 0.82 ^c
9	40	15	OFF	1119 ± 0.18 ^{fg}	4.13 ± 0.007 ^{gh}	15 ± 0.0 ^a	3.46 ± 0.01 ^f	4.2 ± 0.58 ^c
10	40	5	OFF	1080 ± 0.18 ^h	3.88 ± 0.000 ^j	14 ± 0.0 ^b	3.47 ± 0.00 ^e	4.8 ± 1.20 ^a
11	60	15	ON	1481 ± 0.18 ^a	4.71 ± 0.021 ^a	15 ± 0.0 ^a	3.16 ± 0.00 ^h	<0.5 ^m
12	60	10	OFF	1173 ± 0.00 ^e	4.26 ± 0.011 ^{bc}	15 ± 0.0 ^a	3.16 ± 0.06 ^h	3.9 ± 0.70 ^g
13	60	15	OFF	1192 ± 0.18 ^e	4.28 ± 0.003 ^b	15 ± 0.0 ^a	3.17 ± 0.08 ^g	3.8 ± 0.85 ^h
14	40	10	ON	1121 ± 0.18 ^{fg}	4.18 ± 0.011 ^{efg}	15 ± 0.0 ^a	3.54 ± 0.01 ^c	4.3 ± 0.56 ^b
15	40	5	ON	1082 ± 0.18 ^h	4.03 ± 0.007 ⁱ	15 ± 0.0 ^a	3.05 ± 0.00 ⁱ	4.3 ± 0.50 ^a
16	50	15	OFF	1123 ± 0.18 ^{fg}	4.23 ± 0.003 ^{bcde}	15 ± 0.0 ^a	3.88 ± 0.00 ^a	3.9 ± 0.80 ^f
17	50	5	ON	1140 ± 1.80 ^f	4.14 ± 0.018 ^{fgh}	15 ± 0.0 ^a	3.16 ± 0.02 ^h	3.8 ± 0.90 ^h
18	50	10	OFF	1124 ± 1.81 ^{fg}	4.21 ± 0.000 ^{cde}	15 ± 0.0 ^a	3.63 ± 0.05 ^b	4.0 ± 0.40 ^d

X₁ (Temperature, °C), X₂ (Time, min), X₃ (OFF: no US treatment; ON: US treatment). ^{a-m}: Mean with superscripts of different small letters in the same columns are significantly different as Duncan test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3).

ANOVA analysis of the data obtained from the experimental design and the interaction of the temperature, US time, and US power on/off position on the responses for yeast and mold count of samples are shown in Table 5. The results showed that for the yeast and mold content, temperature, time, and ultrasound on/off parameters were statistically significant. Increasing the temperature and processing time reduced yeast and mold count. US power-off which means no ultrasound treatment was used as a control to figure out the effect of the combination of temperature and treatment time used with US power-on position on the results. Under the US power-off condition, temperature application decreased yeast and mold count. Providing the US power-on position and increasing temperature, that process condition made a significant contribution to yeast and mold reduction ($p < 0.05$).

Table 5. ANOVA analysis results of Central Composite Design (CCD) for yeast and mold count in US pasteurization process (log CFU/mL).

Source	Sum of Squares	Mean of Square	F Value	p-Value
Model	15.12	1.89	7.55	0.0033
X ₁	5.07	5.07	20.24	0.0015
X ₂	1.84	1.84	7.35	0.024
X ₃	3.21	3.21	12.81	0.0059
X ₁ X ₂	0.91	0.91	3.64	0.0889
X ₁ X ₃	2.61	2.61	10.43	0.103
X ₂ X ₃	1.14	1.14	4.55	0.0616
X ₁ ²	0.071	0.071	0.28	0.6071
X ₂ ²	0.27	0.27	1.07	0.3289
Residual	2.25	0.25		
Correction Total	17.38			
R ² =0.8703				

X₁ (Temperature, °C), X₂ (min), X₃ (Ultrasound power on/off), $p < 0.05$ indicates statistical significance ANOVA refers to microbial analysis.

As seen in Table 5, the parameters of temperature, time, and the US on/off position were statistically significant ($p < 0.05$) and all parameters tended to increase the amount of TPC. The R² value was 0.9745 for the TPC responses, which was very close to 1 and showed a strong correlation between the independent parameters and responses. The turbidity value, °Brix value, and color a* of US treated samples compared to untreated PJ were not significantly affected by all factors ($p > 0.05$).

Optimization tests were performed for yeast and mold count and TPC amount responses in the design. With the effect of the higher temperature and the US power-on position, yeast and mold count reached to an undetectable level. The increase in temperature and time factors and the contribution of ultrasonic treatment significantly increased the amount of TPC. In addition, turbidity, °Brix, and color a* values were not statistically significant enough to represent the whole experimental design. Therefore, the optimization process was carried out considering the conditions where the yeast and mold content is minimum, and the TPC amount is maximum. Although the best condition to be able to inhibit almost all yeast and mold count was 60 °C, US power on, and 10 min treatment time, the optimum condition was determined that yeast and mold content could be minimized when the temperature was 50 °C, and the treatment time was 15 min, and the US power-on position was applied. Desirability was also found to be 0.802.

3.3. Optimization of Parameters and Design Verification for UV and US Pasteurization

The optimum condition for UV treatment combined with temperature and flow rate were 2 lamps UV, 50 °C and 1.5 L/min, respectively. As for the US treatment, the optimum condition was 50 °C for temperature, 15 min for treatment time, and US power-on position. After determining the optimum condition for UV and US treatment, these two processes were combined and the PJ was examined in terms of the results of TPC, turbidity, °Brix, and color a*, and yeast and mold count. In consideration of stand-alone optimum conditions, the experimental design for the UV+US process was generated to obtain the optimum combined condition. On the other hand, when the experimental design for the UV+US combined process was carried out with 2 lamps, and 1.5 L/min UV application, the responses obtained from yeast and mold count was not meaningful to find the optimum UV+US combined method condition. For this reason, the flow rate was increased to 3.5 L/min and the number of lamps was decreased to 1 lamp considering the responses obtained from the experimental design. In this way, the condition for flow rate and the number of lamp were modified. Under these circumstances, the optimum condition for UV+US was also carried out at different experimental points and evaluated by an expert design program.

The results of the study conducted under these conditions can be observed from the values given in Table 6.

Table 6. Experimental parameters in US+UV pasteurization the observed response values for pomegranate juice.

Run Order	X ₁	X ₂	X ₃	X ₄	X ₅	TPC (mg GAE/L)	Turbidity	°Brix	Color a*	Yeast and Mold (log CFU/mL)
1	40	5	ON	1 LAMP	3.5	1051 ± 3.09 ^a	4.18 ± 0.02 ^a	14 ± 0.0 ^a	3.51 ± 0.09 ^a	3.8 ± 0.0 ^b
2	40	10	ON	1 LAMP	3.5	1067 ± 3.13 ^b	4.23 ± 0.01 ^a	14 ± 0.0 ^a	3.73 ± 0.06 ^b	3.5 ± 0.1 ^b
3	40	15	ON	1 LAMP	3.5	1135 ± 4.17 ^c	4.21 ± 0.05 ^a	14 ± 0.0 ^a	3.53 ± 0.02 ^{ab}	3.1 ± 0.0 ^b
4	50	5	ON	1 LAMP	3.5	1226 ± 2.09 ^d	4.19 ± 0.04 ^a	14 ± 0.0 ^a	3.51 ± 0.09 ^{ab}	2.9 ± 0.1 ^a
5	50	10	ON	1 LAMP	3.5	1244 ± 3.39 ^e	4.21 ± 0.01 ^{ab}	14 ± 0.0 ^a	3.56 ± 0.07 ^{ab}	<0.5 ^c
6	50	15	ON	1 LAMP	3.5	1263 ± 4.11 ^f	4.27 ± 0.02 ^{ab}	15 ± 0.0 ^b	3.35 ± 0.01 ^{ab}	<0.5 ^c
7	60	5	ON	1 LAMP	3.5	1281 ± 1.22 ^g	4.23 ± 0.03 ^c	15 ± 0.0 ^b	3.40 ± 0.05 ^{ab}	<0.5 ^c
8	60	10	ON	1 LAMP	3.5	1289 ± 3.83 ^h	4.38 ± 0.03 ^c	15 ± 0.0 ^b	3.40 ± 0.08 ^{ab}	<0.5 ^c
9	60	15	ON	1 LAMP	3.5	1357 ± 6.26 ⁱ	4.49 ± 0.02 ^d	15 ± 0.0 ^b	3.50 ± 0.00 ^{ab}	<0.5 ^c

X₁ (Temperature, °C), X₂ (Time, min), X₃ (ON: US treatment), X₄ (UV 1 Lamp dose is 5.1 mW/cm²), X₅ (UV Flow rate, L/min). ^{a–i}: Mean with superscripts of different small letters in the same columns are significantly different as Duncan test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3).

Combined US+UV application at 40 °C was ineffective on the reduction of yeast and mold count. The application of 50 °C, and 10 min treatment time reduced the count of yeast and mold to an undetectable level. By applying these conditions, microbial growth was inhibited at lower temperature and treatment time compared with the conventional pasteurization method, and the TPC of PJ was also mostly preserved. Depending on increasing temperature and treatment time, TPC amount of PJ samples significantly increased ($p < 0.05$). Color a* and turbidity values of PJ samples remained almost the same at the different experimental points ($p > 0.05$). As for °Brix results, increasing temperature and treatment time enhanced the °Brix values of PJ samples ($p < 0.05$). In a conclusion, the optimum condition for UV+US combined process was the application of the temperature at 50 °C, UV 1 lamp on, and flow rate 3.5 L/min for 10 min in the US on mode.

3.4. Comparison of UV, US, and UV+US Pasteurization Applications with Conventional Pasteurization

The quality parameters of samples that are untreated and treated with conventional pasteurization, UV, US, and UV+US pasteurization processes are given in Table 7. These values represent the measurements at the optimum point obtained from the separate optimization process. In the conventional method, pasteurization was carried out at 72 °C-15 s. The US, UV, and combined US+UV treatments were applied at 50 °C. For each technique, we used the previously-obtained optimum conditions mentioned above.

Conventional and UV+US pasteurization processes completely exterminated the yeast and mold populations ($p < 0.05$). DPPH radical scavenging activities of PJ treated with UV+US pasteurization process compared to conventional method, the UV + US method retained more DPPH activity ($p < 0.05$). The anthocyanin values of unprocessed and pasteurized samples were in the range of 17.95–25.1 mg/L. The lowest anthocyanin value was obtained from samples applied conventional pasteurization process. The best pasteurization method for saving anthocyanin content in the samples was found to be the US treatment ($p < 0.05$). The HPLC TPC of unprocessed and pasteurized samples differed from 701–1141 µg/g. The TPC amount of unprocessed and pasteurized samples ranged from 889 to 1273 mg GAE/L. The conventional process and UV treatment reduced the TPC amount according to HPLC TPC and TPC analysis results. On the other hand, UV and UV+US treatment increased the amount of phenolic compounds. Turbidity for unprocessed and pasteurized samples was determined in the range of 4.02–4.84 NTU. The lowest and highest value for turbidity were obtained from the UV and conventional methods, respectively. The color a* values of samples did not vary significantly after all

pasteurization methods ($p > 0.05$). As for °Brix value, no significant effect of the UV, US, and UV+US treatment on PJ samples was observed in comparison to PJ applied conventional pasteurization.

Table 7. Variation of the quality parameters of pomegranate juice in different pasteurization conditions vs non-processed.

Quality Parameters	Non-Processed	Conventional	US	UV	US+UV
Anthocyanins (mg/L)	25.1 ± 0.1 ^e	17.95 ± 0.2 ^a	20.6 ± 0.1 ^b	20.85 ± 0.2 ^d	20.7 ± 0.2 ^c
% Inhibition (DPPH)	32.69 ± 0.2 ^e	26.57 ± 0.1 ^a	31.67 ± 0.2 ^d	27.59 ± 0.2 ^c	27.08 ± 0.1 ^b
HPLC TPC (µg/g)	855 ± 5.1 ^b	701 ± 4.9 ^a	1141 ± 7.2 ^e	865 ± 1.2 ^c	1012 ± 3.8 ^d
gallic acid	648.9 ± 0.07 ^b	507.42 ± 0.06 ^a	967.72 ± 0.03 ^e	689.415 ± 0.05 ^c	843.24 ± 0.04 ^d
protocatechuic acid	126.02 ± 0.11 ^e	117.91 ± 0.11 ^d	85.94 ± 0.14 ^b	89.27 ± 0.15 ^c	83.24 ± 0.010 ^a
catechin	38.44 ± 0.13 ^d	24.19 ± 0.10 ^a	27.521 ± 0.01 ^c	27.93 ± 0.10 ^c	25.44 ± 0.08 ^b
<i>p</i> -hydroxybenzoic acid	4.34 ± 0.19 ^a	10.56 ± 0.15 ^b	13.10 ± 0.20 ^d	11.68 ± 0.16 ^c	14.04 ± 0.14 ^e
caffeic acid	11.80 ± 0.10 ^a	13.71 ± 0.13 ^b	15.33 ± 0.15 ^d	15.88 ± 0.13 ^d	14.24 ± 0.09 ^c
myricetin	15.48 ± 0.01 ^a	23.39 ± 0.08 ^b	25.91 ± 0.09 ^c	25.87 ± 0.04 ^c	25.92 ± 0.06 ^c
others	10.02 ± 0.08 ^d	3.82 ± 0.02 ^a	5.479 ± 0.07 ^c	4.955 ± 0.07 ^b	5.88 ± 0.01 ^c
TPC (mg GAE/L)	1078 ± 5.7 ^b	889 ± 4.1 ^a	1273 ± 6.7 ^c	890 ± 7.2 ^a	1263 ± 4.1 ^c
Turbidity	4.13 ± 0.0 ^b	4.84 ± 0.1 ^d	4.26 ± 0.0 ^c	4.02 ± 0.0 ^a	4.27 ± 0.0 ^c
Color a*	3.40 ± 0.0 ^a	3.45 ± 0.0 ^a	3.44 ± 0.0 ^a	3.45 ± 0.0 ^a	3.35 ± 0.0 ^a
°Brix value	14 ± 0.0 ^b	15 ± 0.0 ^a	15 ± 0.0 ^a	15 ± 0.0 ^a	15 ± 0.0 ^a
Yeast and mold count (log CFU/mL)	5.3 ± 0.1 ^d	<0.5 ^a	2.96 ± 0.0 ^b	3.06 ± 0.0 ^c	<0.5 ^a

^{a-e}: Mean with superscripts of different small letters in the same rows are significantly different as Duncan test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3).

4. Discussion

Pomegranate fruit (*Punica granatum* L.) is a source of bioactive compounds such as ellagic acid, punicalagin, and ellagitannins [3,32]. Thermal pasteurization of pomegranate juice generally leads to a decrease in the bioactive compound of fresh juice. The application of UV-C treatment on fresh juice shows that the phenolic content of sample is degraded depending on time and doses [33]. In our study, it was reported that a decrease in the TPC amount of PJ samples was observed compared to unprocessed juice. These results are close to previous research that the UV-C was applied to fresh apple juice [34]. In addition, Pala and Toklucu [35] were reported that a slight decrease occurred in the phenolic content of the pomegranate juice when using high doses of ultraviolet radiation in pasteurization. When the literature studies are examined, it is stated that long-time UV treatment reduces the number of phenolic substances [36]. On the other hand, the lower yeast and mold count was observed by using high doses of UV and longtime UV treatment. The reduction of yeast and mold count varied 1 to 2 log when used different doses and time for exposure. These results have statistically significant similarity with a study conducted by Pala and Toklucu [35] who reported 1 log reduction of yeast and mold count when used 34.4 j/mL and 62.4 j/mL UV. Similarly, the UV-C was applied to grape and cranberry juice inoculated with *Saccharomyces cerevisiae* ATCC 10274 and was reported 0.53 log and 2.5 log reduction [37]. In another study, the UV-C was applied to strawberry juice by Keyser, Müller [19] and the reduction for yeast and mold was found to be 2.45 log CFU/mL.

The °Brix value increased due to the increase in temperature, which is statistically significant ($p < 0.05$) while the effects of other factors (UV dose and flow rate) on the °Brix value of samples are statistically insignificant ($p > 0.05$). It can be explained that an increase in temperature may cause the degradation of dry matter soluble in water and also the amount of water decreases by evaporation and/or the amount of dry matter soluble in water increases proportionally. Our results are similar to the research paper published by Rivas, Rodrigo [38] who mentioned that °Brix value of heat pasteurization applied blended orange and carrot juice increased significantly, compared with the results of untreated juice. With regard to the value of turbidity in pomegranate juice treated by ultraviolet light, we can see that there is a decrease in the value of turbidity, and this decrease is statistically significant. This decrease occurs only when the temperature rises. It can be concluded that

the increase in temperature is able to promote the solubility of dispersed compounds. There are very few studies in the literature that observed the effect of UV irradiation and heat on turbidity. The results of our study are in accordance with these related studies previously conducted on UV irradiation and turbidity [36,39,40].

There are studies that have been conducted on the use of sonication in food products to inhibit *S. cerevisiae* and bacteria by using ultrasound model systems [41–44]. In the present study, the values of TPC for the US pasteurized PJ increased at all experimental points which means all factors (temperature, treatment time, and US power on/off position) were statistically significant. The higher levels of TPC are able to be explained with improvements in the extraction efficiency of the ultrasound process and this process might assist to release of bound form of phenolic acids with the help of the cavitation effect. These results obtained from our study are consistent with other researches [45,46]. The US treatment resulted in an average 1–2 log reduction of the yeast and mold count. Yeast and mold count obtained from at low temperature and the US power-off position were close to each other: The reason is that increasing temperature and the US treatment affect the growth of microorganisms. Our results are close or similar to a previous study that observed the inhibition effect of ultrasound treatment on *Saccharomyces cerevisiae* in pomegranate juice [47]. There were no significant statistical changes in turbidity, °Brix value, and color a^* of US treated samples. These results are similar to a study reported by Tiwari, Patras [48] in red grape juice, and in blackberry juice by Tiwari, O'Donnell [49].

Integrated non-thermal processes offer the potential to reduce the disadvantages of each method and increase the effectiveness of the method [50]. In the present study, the UV and US treatment were combined and the PJ was pasteurized with this integrated method. Since the UV+US treatment more effectively preserved the physicochemical, biochemical, and microbial properties of PJ samples, this method can be used not only fruit juice but also other fluid pasteurization. Compared with the conventional method, our results show that the UV+US treatment enhanced the quality parameters of the pomegranate juice.

5. Conclusions

The expectations of consumers for minimal processing, the nutritional value of food, and healthy food are increasing day by day. Therefore, as an alternative to traditional pasteurization processes, non-thermal treatment options have recently been evaluated more intensively. Two of the most preferred methods are UV and US. In this study, the usage possibilities of UV, US, and combined UV+US methods in pasteurization of pomegranate juice were investigated and compared with the conventional pasteurization process. In addition, an optimization was done to minimize process the conditions using these two non-thermal methods. When the results are examined, it will be seen that US pasteurization was more effective than the UV pasteurization technique for microbial results, and both methods provided great advantages over conventional pasteurization. Furthermore, pasteurization of PJ was more effective when using UV and US methods together whose conditions are 50 °C, UV 1 lamp on, and flow rate 3.5 L/min and for 10 min in the US on mode. It was determined that the pasteurization process could be carried out at lower process conditions. Concerning the integrated use of UV and US systems, it has been concluded that it has great potential for pasteurization of fruit juices.

Author Contributions: T.A.M.A. obtained the results, data, analysis, and interpretation, and drafted the manuscript based on the results. M.Z.D., N.C.I. and G.U.O. planned with the concept design, study, and review of the manuscript regarding the important intellectual content and final approval of the copy, and those four authors verified it. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Aviram, M.; Dornfeld, L.; Rosenblat, M.; Volkova, N.; Kaplan, M.; Coleman, R.; Hayek, T.; Presser, D.; Fuhrman, B. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: Studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *Am. J. Clin. Nutr.* **2000**, *71*, 1062–1076. [CrossRef] [PubMed]
2. Borochov-Neori, H.; Judeinstein, S.; Tripler, E.; Harari, M.; Greenberg, A.; Shomer, I.; Holland, D. Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. *J. Food Compos. Anal.* **2009**, *22*, 189–195. [CrossRef]
3. Gil, M.I.; Tomás-Barberán, F.A.; Hess-Pierce, B.; Holcroft, D.M.; Kader, A.A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem.* **2000**, *48*, 4581–4589. [CrossRef] [PubMed]
4. Alighourchi, H.; Barzegar, M. Some physicochemical characteristics and degradation kinetic of anthocyanin of reconstituted pomegranate juice during storage. *J. Food Eng.* **2009**, *90*, 179–185. [CrossRef]
5. Patras, A.; Brunton, N.P.; O'Donnell, C.; Tiwari, B. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food Sci. Technol.* **2010**, *21*, 3–11. [CrossRef]
6. Tournas, V.; Heeres, J.; Burgess, L. Moulds and yeasts in fruit salads and fruit juices. *Food Microbiol.* **2006**, *23*, 684–688. [CrossRef]
7. López-Rubira, V.; Conesa, A.; Allende, A.; Artés, F. Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biol. Technol.* **2005**, *37*, 174–185. [CrossRef]
8. Adekunle, A.; Tiwari, B.; Cullen, P.; Scannell, A.; O'donnell, C. Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chem.* **2010**, *122*, 500–507. [CrossRef]
9. Putnik, P.; Kresoja, Ž.; Bosiljkov, T.; Jambrak, A.R.; Barba, F.J.; Lorenzo, J.M.; Roohinejad, S.; Granato, D.; Žuntar, I.; Kovačević, D.B. Comparing the effects of thermal and non-thermal technologies on pomegranate juice quality: A review. *Food Chem.* **2019**, *279*, 150–161. [CrossRef]
10. Plaza, L.; Sánchez-Moreno, C.; Elez-Martínez, P.; de Ancos, B.; Martín-Belloso, O.; Cano, M.P. Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. *Eur. Food Res. Technol.* **2006**, *223*, 487–493. [CrossRef]
11. Knorr, D.; Zenker, M.; Heinz, V.; Lee, D.-U. Applications and potential of ultrasonics in food processing. *Trends Food Sci. Technol.* **2004**, *15*, 261–266. [CrossRef]
12. Piyasena, P.; Mohareb, E.; McKellar, R. Inactivation of microbes using ultrasound: A review. *Int. J. Food Microbiol.* **2003**, *87*, 207–216. [CrossRef]
13. Cheng, L.; Soh, C.; Liew, S.; Teh, F. Effects of sonication and carbonation on guava juice quality. *Food Chem.* **2007**, *104*, 1396–1401. [CrossRef]
14. Caminiti, I.M.; Palgan, I.; Muñoz, A.; Noci, F.; Whyte, P.; Morgan, D.J.; Cronin, D.A.; Lyng, J.G. The effect of ultraviolet light on microbial inactivation and quality attributes of apple juice. *Food Bioprocess Technol.* **2012**, *5*, 680–686. [CrossRef]
15. Franz, C.M.; Specht, I.; Cho, G.-S.; Graef, V.; Stahl, M.R. UV-C-inactivation of microorganisms in naturally cloudy apple juice using novel inactivation equipment based on Dean vortex technology. *Food Control* **2009**, *20*, 1103–1107. [CrossRef]
16. National Advisory Committee Onmicrobiological Criteria for Foods. Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization. *J. Food Prot.* **2006**, *69*, 1190–1216. [CrossRef]
17. Koutchma, T. Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food Bioprocess Technol.* **2009**, *2*, 138–155. [CrossRef]
18. FDA. Available online: <https://www.fda.gov/drugs/cder-biomarker-qualification-program/list-qualified-biomarkers> (accessed on 25 May 2020).
19. Keyser, M.; Müller, I.A.; Cilliers, F.P.; Nel, W.; Gouws, P.A. Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 348–354. [CrossRef]

20. Fonteles, T.V.; Costa, M.G.M.; de Jesus, A.L.T.; de Miranda, M.R.A.; Fernandes, F.A.N.; Rodrigues, S. Power ultrasound processing of cantaloupe melon juice: Effects on quality parameters. *Food Res. Int.* **2012**, *48*, 41–48. [\[CrossRef\]](#)
21. Tiwari, B.; Muthukumarappan, K.; O'donnell, C.; Cullen, P. Colour degradation and quality parameters of sonicated orange juice using response surface methodology. *LWT–Food Sci. Technol.* **2008**, *41*, 1876–1883. [\[CrossRef\]](#)
22. Chandrapala, J.; Oliver, C.; Kentish, S.; Ashokkumar, M. Ultrasonics in food processing. *Ultrason. Sonochemistry* **2012**, *19*, 975–983. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Awad, T.; Moharram, H.; Shaltout, O.; Asker, D.; Youssef, M. Applications of ultrasound in analysis, processing and quality control of food: A review. *Food Res. Int.* **2012**, *48*, 410–427. [\[CrossRef\]](#)
24. Su, D.; Xiao, T.; Gu, D.; Cao, Y.; Jin, Y.; Zhang, W.; Wu, T. Ultrasonic bleaching of rapeseed oil: Effects of bleaching conditions and underlying mechanisms. *J. Food Eng.* **2013**, *117*, 8–13. [\[CrossRef\]](#)
25. Muñoz, A.; Palgan, I.; Noci, F.; Morgan, D.; Cronin, D.; Whyte, P.; Lyng, J. Combinations of high intensity light pulses and thermosonication for the inactivation of *Escherichia coli* in orange juice. *Food Microbiol.* **2011**, *28*, 1200–1204. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Tiwari, B.; Muthukumarappan, K.; O'Donnell, C.; Cullen, P. Effects of sonication on the kinetics of orange juice quality parameters. *J. Agric. Food Chem.* **2008**, *56*, 2423–2428. [\[CrossRef\]](#)
27. Nafar, M.; Emam-Djomeh, Z.; Yousefi, S.; Hashemi Ravan, M. An optimization study on the ultrasonic treatments for *saccharomyces cerevisiae* inactivation in red grape juice with maintaining critical quality attributes. *J. Food Qual.* **2013**, *36*, 269–281. [\[CrossRef\]](#)
28. Ngo, T.H.D.; Ngo, D.N. Effects of low-frequency ultrasound on heterogenous deacetylation of chitin. *Int. J. Biol. Macromol.* **2017**, *104*, 1604–1610. [\[CrossRef\]](#)
29. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
30. Wrolstad, R.E.; Durst, R.W.; Lee, J. Tracking color and pigment changes in anthocyanin products. *Trends Food Sci. Technol.* **2005**, *16*, 423–428. [\[CrossRef\]](#)
31. Durak, M.Z.; Ucak, G. Solvent optimization and characterization of fatty acid profile and antimicrobial and antioxidant activities of Turkish *Pistacia terebinthus* L. extracts. *Turk. J. Agric. For.* **2015**, *39*, 10–19. [\[CrossRef\]](#)
32. Fischer, U.A.; Carle, R.; Kammerer, D.R. Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MSn. *Food Chem.* **2011**, *127*, 807–821. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Shamsudin, R.; Adzahan, N.M.; Yee, Y.P.; Mansor, A. Effect of repetitive ultraviolet irradiation on the physico-chemical properties and microbial stability of pineapple juice. *Innov. Food Sci. Emerg. Technol.* **2014**, *23*, 114–120. [\[CrossRef\]](#)
34. Noci, F.; Riener, J.; Walkling-Ribeiro, M.; Cronin, D.; Morgan, D.; Lyng, J. Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. *J. Food Eng.* **2008**, *85*, 141–146. [\[CrossRef\]](#)
35. Pala, Ç.U.; Toklucu, A.K. Effect of UV-C light on anthocyanin content and other quality parameters of pomegranate juice. *J. Food Compos. Anal.* **2011**, *24*, 790–795. [\[CrossRef\]](#)
36. Orłowska, M.; Koutchma, T.; Kostrzyńska, M.; Tang, J.; Defelice, C. Evaluation of mixing flow conditions to inactivate *Escherichia coli* in opaque liquids using pilot-scale Taylor–Couette UV unit. *J. Food Eng.* **2014**, *120*, 100–109. [\[CrossRef\]](#)
37. Guerrero-Beltrán, J.; Welti-Chanes, J.; Barbosa-Cánovas, G.V. Ultraviolet-C light processing of grape, cranberry and grapefruit juices to inactivate *Saccharomyces cerevisiae*. *J. Food Process Eng.* **2009**, *32*, 916–932. [\[CrossRef\]](#)
38. Rivas, A.; Rodrigo, D.; Martinez, A.; Barbosa-Cánovas, G.; Rodrigo, M. Effect of PEF and heat pasteurization on the physical-chemical characteristics of blended orange and carrot juice. *LWT–Food Sci. Technol.* **2006**, *39*, 1163–1170. [\[CrossRef\]](#)
39. Tandon, K.; Worobo, R.W.; Churey, J.J.; Padilla-Zakour, O.I. Storage quality of pasteurized and UV treated apple cider. *J. Food Process. Preserv.* **2003**, *27*, 21–35. [\[CrossRef\]](#)
40. Chia, S.; Rosnah, S.; Noranizan, M.; Ramli, W.W. The effect of storage on the quality attributes of ultraviolet-irradiated and thermally pasteurised pineapple juices. *Int. Food Res. J.* **2012**, *19*, 1001.

41. Bermúdez-Aguirre, D.; Barbosa-Cánovas, G.V. Inactivation of *Saccharomyces cerevisiae* in pineapple, grape and cranberry juices under pulsed and continuous thermo-sonication treatments. *J. Food Eng.* **2012**, *108*, 383–392. [[CrossRef](#)]
42. Char, C.D.; Mitilinaki, E.; Guerrero, S.N.; Alzamora, S.M. Use of high-intensity ultrasound and UV-C light to inactivate some microorganisms in fruit juices. *Food Bioprocess Technol.* **2010**, *3*, 797–803. [[CrossRef](#)]
43. Guerrero, S.; López-Malo, A.; Alzamora, S. Effect of ultrasound on the survival of *Saccharomyces cerevisiae*: Influence of temperature, pH and amplitude. *Innov. Food Sci. Emerg. Technol.* **2001**, *2*, 31–39. [[CrossRef](#)]
44. Guerrero, S.; Tognon, M.; Alzamora, S.M. Response of *Saccharomyces cerevisiae* to the combined action of ultrasound and low weight chitosan. *Food Control* **2005**, *16*, 131–139. [[CrossRef](#)]
45. Abid, M.; Jabbar, S.; Wu, T.; Hashim, M.M.; Hu, B.; Lei, S.; Zhang, X.; Zeng, X. Effect of ultrasound on different quality parameters of apple juice. *Ultrason. Sonochemistry* **2013**, *20*, 1182–1187. [[CrossRef](#)]
46. Zafra-Rojas, Q.Y.; Cruz-Cansino, N.; Ramírez-Moreno, E.; Delgado-Olivares, L.; Villanueva-Sánchez, J.; Alanís-García, E. Effects of ultrasound treatment in purple cactus pear (*Opuntia ficus-indica*) juice. *Ultrason. Sonochemistry* **2013**, *20*, 1283–1288. [[CrossRef](#)] [[PubMed](#)]
47. Alighourchi, H.; Barzegar, M.; Sahari, M.A.; Abbasi, S. The effects of sonication and gamma irradiation on the inactivation of *Escherichia coli* and *Saccharomyces cerevisiae* in pomegranate juice. *Iran. J. Microbiol.* **2014**, *6*, 51.
48. Tiwari, B.; Patras, A.; Brunton, N.; Cullen, P.; O'donnell, C. Effect of ultrasound processing on anthocyanins and color of red grape juice. *Ultrason. Sonochemistry* **2010**, *17*, 598–604. [[CrossRef](#)]
49. Tiwari, B.; O'Donnell, C.; Cullen, P. Effect of sonication on retention of anthocyanins in blackberry juice. *J. Food Eng.* **2009**, *93*, 166–171. [[CrossRef](#)]
50. Kilicli, M.; Baslar, M.; Durak, M.Z.; Sagdic, O. Effect of ultrasound and low-intensity electrical current for microbial safety of lettuce. *LWT* **2019**, *116*, 108509. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).