REPUBLIC OF TURKEY

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

IMPROVEMENT OF THE FUNCTIONAL PROPERTIES OF POMEGRANATE JUICE BY ULTRAVIOLET, ULTRASOUND TREATMENT AND PROBIOTIFICATION

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DOCTOR OF PHILOSOPHY THESIS

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December, 2020

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ACKNOWLEDGEMENTS

A special thanks to Assoc. Prof. Dr. Muhammed Zeki DURAK, my advisor for his countless hours of reflecting, reading, encouraging, and most of the patience throughout the entire process.

I want to thank the jury members who had devoted their valuable time and efforts to my thesis and for agreeing to serve on my committee.

I want to acknowledge and thank the faculty members and assistants of the food engineering department at Yildiz Technical University for allowing me to conduct my research and provide feedback that made the completion of this research possible.

Finally, I would not be remiss in thanking the Iraqi Ministry of Higher Education and Scientific Research for providing me a full time-scholarship (2015-2020) to accomplish the requirements of my graduate studies (Ph.D.) in an esteemed university, YTU. Above anything else, I would thank my entire family for their supports, solidarity, and patience during my studies here in Turkey.

Tareq Abdulrazzaq ALABDALI

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LIST OF SYMBOLS

°C	Celsius degree
F	Frequency
g	Gram
Hz	Hertz
kHz	Kilo Hertz
L	Liter
mg	Milligram
mL	Milliliter
min	Minute
NTU	Nephelometric turbidity unit
%	Percentage
P	Power (W)
pH	Power of Hydrogen
sec	Second
p	Statistical significance

LIST OF ABBREVIATIONS

$AgNO_3$	Silver nitrate
$AlCl_3$	Aluminum Trichloride
CFU	Colony-forming unit
DPPH	2,2-diphenyl-1-pcyrylhydrazyl
FCR	Folin- Ciocalteu reagent
FDA	Food and Drug Administration
FPJ	Fermented pomegranate juice
GAE	Gallic acid equivalent (mg/L)
HCl	Hydrochloric acid
K_2CrO_4	Potassium chromate
LAB	Lactic acid bacteria
log	Logarithm
MIA	Ingredient analysis
NaClO	Sodium hydrochloride
PCA	Plate Count Agar
Spp	Subspecies
TEAC	Trolox equivalent antioxidant capacity measurement
TMAB	Total mesophilic aerobic bacteria
TPC	Total phenolic acid
UI	Ultrasound intensity
US	Ultrasound
UV	Ultraviolet

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ABSTRACT

Improvement of The Functional Properties of Pomegranate Juice by Ultraviolet, Ultrasound Treatment and Probiotification

Tareq Abdulrazzaq ALABDALI

Department of Food Engineering

Doctor of Philosophy Thesis

Advisor: Assoc. Prof. Muhammed Zeki DURAK

This research aims to determine the combined usage possibilities of ultraviolet (UV) and ultrasonic (US) processes in the pasteurization of pomegranate juice and production of the functional pomegranate juice by fermented the final optimum point from pasteurization pomegranate juice with bacteria *Lactobacillus plantarum*. For this purpose, UV, US, and combined UV+US pasteurization of pomegranate juice were optimized using experimental designs, such as the central composite design and factorial design, and compared with the conventional pasteurization process. Total phenolic content, color a*, water-soluble dry matter (°Brix), turbidity, anthocyanin, DPPH, HPLC TPC, and yeast and mold count and were used as quality parameters during all of the processes. The results showed that the application of 50 °C, a 3.5 L/min flow rate, and 5.1 mW/cm^2 UV dose, and 10 min US (200 Watt) reduced the microbial population below the detection limits. *Lactobacillus plantarum* was taken previously identified by genotypic characterization of the bacteria genomic DNA by PCR technique [1]. The *Lactobacillus plantarum* was added to pomegranate juice pasteurized by combined pasteurization. During four weeks' storage at 5 °C, the enumeration of

Lactobacillus plantarum and physicochemical and bioactive probiotic pomegranate juice properties were observed. Total phenolic content and antioxidant activity were greater in the fermented pomegranate juice than in unfermented juice after 24 h fermentation and for 28 days. The probiotic bacteria were valued after 24 hours of fermentation 9.64 log CFU/mL, in fermented juicy and viability of the probiotic bacteria remains over ~7.11 log CFU/mL the length of the storage period for four weeks. No growth of yeasts and mold were observed in the fermented pomegranate beverage throughout preservation time.Incorporating UV + US processes into the pasteurization process can reduce microbial activity at lower temperatures and times than the conventional pasteurization process, thus preserving the bioactive compounds present in juices.

Keyword: Ultraviolet irradiation, Ultrasound, *Lactobacillus plantarum,* Pomegranate juice, Functional beverage

ÖZET

Nar Suyunun Fonksiyonel Özelliklerinin Ultraviolet, Ultrason Arıtma ve Probiyotifikasyon ile Gelişme

Tareq Abdulrazzaq ALABDALI

Gıda Mühendisliği Anabilim Dalı

Doktora Tezi

Danışman: Doç. Dr. Muhammed Zeki DURAK

Araştırma, nar suyunun pastörizasyonundan elde edilen son optimum noktayı *Lactobacillus plantarum* bakterileri ile fermente ederek, nar suyunun pastörizasyonu ve fonksiyonel nar suyunun üretiminde ultraviyole (UV) ve ultrasonik (US) işlemlerinin kombine kullanım olanaklarını belirlemeyi amaçlamaktadır. Bu amaçla, nar suyunun UV, US ve kombine UV + US pastörizasyonu, merkezi kompozit tasarım ve faktöriyel tasarım gibi deneysel tasarımlar kullanılarak optimize edilmiş ve geleneksel pastörizasyon süreci ile karşılaştırılmıştır. Toplam fenolik içerik, renk a *, suda çözünebilen kuru madde (° Brix), bulanıklık, antosiyanin, DPPH, HPLC TPC ve maya ve küf sayımı tüm proseslerde kalite parametresi olarak kullanılmıştır. Sonuçlar 50 °C, 3.5 L / dak akış hızı ve 5.1 mW / cm2 UV dozu ve 10 dak US (200 Watt) uygulamasının mikrobiyal popülasyonu saptama limitlerinin altına düşürdüğünü gösterdi. *Lactobacillus plantarum*, daha önce PCR tekniği ile bakteri genomik DNA'sının genotipik karakterizasyonu ile tanımlanmıştı [1]. *Lactobacillus plantarum*, kombine pastörizasyonla pastörize edilen nar suyuna ilave edildi. 5 °C'de dört haftalık saklama sırasında, *Lactobacillus plantarum* sayımı ve fizikokimyasal ve

biyoaktif probiyotik nar suyu özellikleri gözlendi. Toplam fenolik içerik ve antioksidan aktivite, fermente edilmiş nar suyunda, 24 saatlik fermantasyondan sonra ve 28 gün boyunca fermente edilmemiş meyve suyundan daha fazlaydı. Probiyotik bakteriler 24 saatlik fermentasyondan sonra 9,64 log CFU / mL, fermente sulu olarak değerlendirildi ve probiyotik bakterilerin canlılığı, dört hafta boyunca saklama süresi boyunca -7,11 log CFU / mL'nin üzerinde kaldı. Fermente edilmiş nar suyunda depolama süresi boyunca maya ve küf büyümesi gözlenmemiştir.

UV + US işlemlerinin pastörizasyon işlemine dahil edilmesi, mikrobiyal aktiviteyi geleneksel pastörizasyon işleminden daha düşük sıcaklıklarda ve sürelerde azaltabilir, böylece meyve sularında bulunan biyoaktif bileşikleri koruyabilir.

Anahtar Kelime: Ultraviyole ışınlama, Ultrason, *Lactobacillus plantarum*, Nar suyu, Fonksiyonel içecek

1
INTRODUCTION

1.1 Literature Review

In recent years, food manufacturers have tended to search for alternative ways to pasteurize juices and drinks instead of traditional pasteurization, known as heat pasteurization, to avoid losses from the nutritional value resulting from excessive heat ensure the juice is free from microbial contamination. The trend has been towards the use of non-thermal methods in pasteurizing juices and drinks. The consumer's requirements have also increased in recent times to search for a product rich in antioxidants and phenolic compounds and with a high nutritional value content, which has excellent benefits that promote human health.

To keep pace with consumer requirements, many studies and research have been carried out on fermentation of juices with *Lactobacillus* bacteria to increase their nutritional value, increase their total phenolic content and antioxidant and improve their taste.

Our study focused on using ultraviolet rays and ultrasound in pomegranate juice pasteurization and the fermentation of pomegranate pasteurized beverage with *Lactobacillus plantarum* bacteria as a tool to improve total phenolic content and antioxidant activity for fermented juice.

Although ultraviolet radiation (UV) is a commonly used non-thermal technique for pasteurizing fruit juices, few studies have used ultraviolet light in pomegranate juice pasteurization, including Ç. U. Pala [2]. He treated the pomegranate juice extracted from the grains with ultraviolet rays using different doses as the doses ranged between 12.5 to 62.4 J/mL ultraviolet radiation dose. The pomegranate juices, pasteurized by ultraviolet radiation, maintained the quality of the pomegranate juice. They preserved a large percentage of the juice anthocyanin content compared to the thermal pasteurization temperature of 90 °C for two minutes.

Where a decrease in anthocyanin content were observed between 8.1% and 16.3%. Where pasteurization with ultraviolet rays reduced the number of yeasts, mold, and aerobic bacteria, and also the number of *Escherichia coli* bacteria that stain ATCC 25922 were reduced as follows, as it reduced the number of *E. coli* bacteria to 6.15 CFU/mL, yeasts and mold to 1.45 CFU/mL, and aerobic bacteria to 1.8 CFU/mL in pasteurized pomegranate juice. The results obtained in this study proved that using ultraviolet rays of pomegranate juice to reduce the number of harmful microorganisms and extend pomegranate juice storage life when stored at a temperature between (4-10 °C) without changing the nutritional properties of the juice.

On the other hand, a study were used, UV rays were used to pasteurize fresh white grape juice. The juice flow rate in the device were 0.90 mL/s, as it were cycled eight times in the ultraviolet ray device; the UV exposition time for the juice were 244 s per cycle and the processing time were 32 min. As the result of the treatment were the reduction number of *E. coli* K-12 bacteria by 5.34 log cycles after juice treatment, were exposed juice to a dose of ultraviolet radiation by an amount 9.92 J/cm^2 were flow rate in the device were 0.90 mL/s. Due to its ultraviolet light treatment, white grape juice validity period were prolonged to 14 days at 4 °C. This study has not been shown that UV treatment affects the total soluble solids, pH value, and titratable acidity in white grape juice. This study showed that the treatment with ultraviolet radiation significantly affects color, turbidity, absorption factor, and ascorbic acid content. Moreover, all physical and chemical properties of juice were changed through cryogenic storage. Whereas after the pasteurization using ultraviolet radiation, the expiry date of fresh turbid white grape juice were increased, while that treatment affected similarly negatively in the control samples [3].

Moreover, a research paper published on ultraviolet radiation and thermal pasteurization on lemon juice and melon juice. He examined UV-C their effect on *Escherichia coli* K12 that strain is ATCC 25253 and analyzed some analysis like total phenolic and Brix Value, turbidity, titratable acidity both immediately and after 30 days storage.

The newly designed lemon juice and melon juice mixture include 12 percent the value were v/v of lemon juice that were the pH value of the juice were 3.92 ± 0.02 had been the high result in the customers' approval test. The dose of ultraviolet radiation used in the juice pasteurization were 2.462 J/mL. The degree of thermal pasteurization used were 72 °C for 71 seconds, where decreases for *Escherichia coli* K12 in lemon juice and melon juice mixture were <as to less than 6 log CFU/mL. The results showed that the analysis of the main ingredients analysis (MIA) in the juice showed clear differences in the juice physical and chemical properties compared to ultraviolet and thermal pasteurization.

The lemon juice and melon juice mixture through storage time indicate that ultraviolet is better in terms of inhibiting microbes than thermal pasteurization when the juice is stored at 4 °C. The stability of the shelf life of pasteurized melon and lemon juice blends were assessed by main ingredients analysis (MIA) and physical-chemical analysis and microbial analysis. As the result of treating the juice mixtures of lemon juice and watermelon by the two pasteurization methods were to increase the juice shelf life from 2 to 30 days. Ultraviolet radiation has better for inactivation of the microbial when stored at 4 °C. The pasteurization by ultraviolet radiation were better in preserving the quality of juice than thermal pasteurization in both fresh juices or the juice mixture of lemon juice and watermelon juice. This will be an essential feature of juices' pasteurization and preserving nutritional value [4].

Moreover, there were research prepared by M. Chisari [5] it about the impact of UV irradiation on fresh cat melon were investigated, and the dose were used 254 nm, 0.04 kJs^{-1} m^2 the time used for pasteurization were 30 and 60 and 120 seconds. Its effect on the activities of major degraded enzymes such as pectin, peroxidase, polyphenol oxidase, and methylesterase and effect on color through stockpiling at 5 °C. The enzymatic activities were similar to samples washed with water and treated with ultraviolet rays and samples washed with 100 mg 1 NaOCl and much less than untreated samples, especially after storing them for seven days at 5 °C. However, UV curing were 7 to 12% stronger from untreated samples. The lowest perceptible color variety expressed as ΔE* were in the sample exposed to

UV rays 120-second radiation were ΔE = 8.58. At the same time, the elevated value were noticed in the untreated sample ΔE = 11.06.

On the other hand, in research prepared in 2013, were used samples of orange juice purposely fermented to improve natural microflora that were primarily yeasted and consequently subjected to UV-C radiation with a power of 1.32 mW/ cm^2 and a specimen depth of 0.153 cm multiple periods with a parallel radiation beam.

If the ultraviolet dose were not at the first load of 0 and 108.42 mJ / cm^2, the ANOVA examination showed that the exposure time also significantly influenced the normal flora's inactivation from that orange juice ($p<0.0001$). Analysis by Tukey demonstrated that the increase in ultraviolet ray irradiation leads to a significant decrease log viability. The highest log-lowering in yeast and mold counts were achieved in doses of 108.42 mJ / cm^2 of UV 1.76 log10 in 20 min ultraviolet ray irradiation (I0= 1.32 mW / cm^2) [6].

Tran and Farid [7], through the results gained from the research, where the use of ultraviolet rays in the treatment of orange juice, where the dose of ultraviolet rays that used to inhibit yeasts and mold and aerobic bacteria is 87±7 and 119±17 mJ/cm^2. When using the (73.8 mJ/cm^2) dose of UV rays, it can extend the shelf life of orange juice to five days. The deterioration of vitamin C were examined when using thermal pasteurization or using ultraviolet rays at a dose of 100 mJ/cm^2, which were the same when two methods are used. There were no significant changes in vitamin C value. The treatment did not significantly influence the color and pH of the juice.

There is a study prepared on the use of ultraviolet radiation on fresh-cut melon juice and the effect of this technique on the microbial content and the physicochemical characteristics of the juice. As the juice were exposed to ultraviolet radiation, it reduced 2 logs for both total viable count and Enterobacteriaceae compared to untreated juice. The ultraviolet radiation did not have a clear effect on the juice color stability during the treatment or the juice storage. The use of ultraviolet rays resulted in the appearance of a good taste that

made it highly preferred over untreated samples [72]. UV-C light has been successfully applied to fresh-cut melon as a new technology to disinfect surfaces.

Ultrasound 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 [9], [10].

Pala et al. (2015) [11] however, it were found that pomegranate juice pasteurized by ultrasound were able to reduction about 5-log of *E. coli* ATCC 25922 bacteria and bacteria *E. coli* O157:H7 that injected into pomegranate juice, which meets international Food and Drug Administration standards to reduce pathogens in juices and drinks. With the same conditions of the operation, ultrasound could inhibit the *S. cerevisiae* by approximately 1.36-log. Besides, pressure and temperature are required to inhibit yeasts and mold in pomegranate juice pasteurized by ultrasound.

Monomeric anthocyanins' content in pomegranate juice were stable during the ultrasound treatment of the juice at 50% strength up to 30 minutes of treatment and at 75 and 100% ultrasound strengths up to 24 and 18 minutes, accordingly, result in non-meaningful reduction ($P > 0.05$). However, a decrease in anthocyanin concentration observed at high ultrasound amplitudes at 75 and 100%, and treatment time were >18 minutes. The ability to retain the monomeric anthocyanin concentration were 12 minutes after the ultrasound treatment 98.5% (275.54 to 271.36 mg/L), 97.3% (270.11 to 262.80 mg/L), and 95.9% (283.46 to 271.77 mg/L) at 50, 75, and 100% amplitudes, respectively. It were found that the color during pasteurization was influenced by the amplitude of the ultrasound waves and the processing time, and visible color discrepancies occurred through an extreme processing environment. However, the total phenol content did not change significantly. In general, this study indicates that ultrasound technology can improve pomegranate juice quality depending on different treatment times

and energy levels. Nonsignificant change in pH and °Brix of all treatments with different amplitudes of the US.

Anthocyanin degradation during ultrasound processing of pomegranate juice follows the primary -order kinetics higher where amplitude and longest ultrasonic time adversely changed mono anthocyanin content in pomegranate juice. The deterioration of anthocyanins may be explained by the cavitation breakdown of microscopic bubbles and the generation of oxidative produce like free radicals. PALA et al., 2015 found that ultrasound uses were one of the significant critical factor that affect anthocyanin stable through ultrasonication.

A study used ultrasound to pasteurize pomegranate juice, and the strength used for ultrasound were 50 - 75 - 100, and the treatment time were 0-3-6-9 min. The results showed that the ultrasound process had no important impact on the pH value, acidity, °Brix value. The percentage of total anthocyanin value were deteriorating were 0.38-9. 75%. The total amount has increased by 0.44-7.32% at several magnitude levels and times. The total phenol value were also increased in several ultrasound-treated juice samples, 5.40-42.52% at 100% intensities, and 9 min.

Moreover, the antioxidant activity did not significantly change compared to the control sample ($p < 0.01$). Find that after ultrasound treatment for pomegranate juice, the overall content of anthocyanins has decreased. This decrease could be caused by microscopic cavitation collapses and the production of an oxidation substance that could affect the anthocyanin's stability. The outcome were noted in those same studies that the total phenolic value of ultrasound-pasteurized pomegranate juice increases; this increase in cloudy juice might likely compare to pure juices. The reason for more efficient extraction may be that the increase in the number of phenols from cloudy particles results from treating the juice by ultrasound [12].

This study demonstrated that the power of ultrasound when 50 - 75 % of energy to inhibit microorganisms were not significant. In comparison, the reduction of microorganisms significantly were observed when using 100% energy with 15 minutes of treatment, which decreased *E. coli* and *S. cerevisiae* populations. by

3.37-3.56 log CFU/mL and 1.84-1.88 log CFU/mL, respectively. Low pH of pomegranate juice has a significant effect on inhibiting bacteria *E. coli* or yeast-like *S. cerevisiae*. Simultaneously, the low pH of pomegranate juice were not a significant changes in bacteria *E. coli* and *S. cerevisiae* pasteurized by ultrasound [13].

In a study, ultrasound were used on the blackberry juice to see how this treatment affected the juice color value and the anthocyanin value. The ultrasound's strength used 40% to 100% and time sonication were 0–10 min, and the constant frequency were 20 kHz. Where significant retention in the anthocyanin content (>94%) were observed when using ultrasound power in the most extreme conditions 100% of the treatment for 10 minutes, indicating that the anthocyanin stabilized during the acoustic treatment. The color value changes were very slight during the acoustic treatment of the juice [14]. Feng& Yang (2011) showed that gram-positive cells and *cocci* cells are most resistive to inhibition through ultrasound from vegetative and Gram-negative bacteria and rod- formed bacteria.

Ultrasound treatment of fruit juice has been reported to enhance the period of validity with minimum adverse impact upon the quality of fruit juices while reducing processing costs compared to conventional heat treatment [15]

Moreover, the low-frequency ultrasound with moderate temperature helps reduce the treatment heat and time at 16 and 55%, severally. Therefore, it has been determined as a hopeful resolution to fruit juices' pasteurization [16].

The flowing study observed that the total phenolic value of ultrasound-pasteurized pomegranate juice increases; this increase in cloudy juice is might likely compare to pure juices. It may be based on the better effective extraction of cloudy particles' phenols through the ultrasound process [12].

Tuğba Türken and Hande S Erge [17] published a paper on the action of ultrasound on sour cherry drink under different levels of amplitude (50, 75, 100%) and under different temperatures (20, 30, 40 °C) and three different times of treatment 2, 6, 10 min and at a steady frequency of 20 kHz. The ultrasound had no important impact on the pH, Brix value, and titratable acidity. The total monomeric anthocyanin content's significant effect were detected at increased

ultrasound waves' amplitudes ($p<0.01$). Higher amplitudes of ultrasound increased the total phenol content detected upon increasing the temperature ($p<0.05$). The antioxidants had an important significant ($p<0.05$) impact on increasing the ultrasound's different amplitudes. The color parameters (L *, a *, b *, C, h) increased in general by increasing the temperature, capacity level, and curing time *E. coli* O157: H7 were shown to significantly impact heating and treatment time ($p <0.05$).

Regarding the fermentation of pomegranate juice to produce juice that contains probiotic bacteria, some studies have been conducted on pomegranate juice. Some reports fermenting pomegranate drinks (FP) or pomegranate wine could benefit good human health. It is common knowledge that through study, that the fermentation method could raise the digestibility and bioavailability of the bioactive ingredients present in those drinks [18]. The advantageous property is attributed to the antioxidants present in those beverages [19]. Additionally, fermented pomegranate juice is a significant antioxidant source like anthocyanins, delphinidin, 3-O-glucoside, and 3,5-O-diglucoside from cyanidin and pelargonidin[20].

Several studies have shown that pomegranate juice fermented by lactic acid bacteria, filamentous fungi, or yeasts is a promising tool to improve its nutritional value and functional property further [18], [21], [22]. Fermentation with selected lactic acid bacteria enabled improved antioxidant activity, expiry date, and organoleptic property of pomegranate juice [23]. Besides the metabolic conversion of phenolic compounds by lactic acid bacteria as an effective detoxification mechanism [24]. Fermentation results in strong souring with an associated advanced bioactive content unleash, increasing bio-accessibility, and bioavailability [25].

This survey were conducted to develop a fermented pomegranate drink-through used probiotic lactic acid bacteria and examine the fermented pomegranate drinks' shelf life and biochemical property. Pomegranate juice lonely and mixed with various proportions of kokum juice were vaccinated 24 hours with lactic acid bacteria and incubating at 37 °C for 72 hours. The results indicated that

pomegranate juice fermented with and without kokum juice fermenting through lactic acid bacteria decreased the pH and increased the acidity, antioxidant activity, and total phenol content. The number of lactic acid bacteria decreased throughout the storage duration within the fermentation beverage. Overall acceptance through a sensory/sensory assessment of fermenting pomegranate about the nine-point pleasure scale showed that drinks fermented with a 15% mixture of kokum juice showed the highest unfertilized drinks pomegranate juice7.55 out of 10 [26].

Moreover, the researcher undertook [27] by fermentation of pomegranate juice by using four strains from lactic acid bacteria: *L. delbruekii*, *L. acidophilus*, *L. paracasei Lactobacillus plantarum*, and storage at 4 °C through 4 weeks to analyzed the growth of microbial, sugar, acid value, titrable acidity and organic acid metabolic have been measured through the fermenting. The results were *L. delbruekii* and *L. Plantarum* sharply increased pH at the initial fermenting phases. Consumption of sugar were also higher than other strains, and for the two strains during fermentation, there were better microbial growth. As the principal organic acid in pomegranate juice, citric acid were mainly eaten by all probiotic lactic acid bacteria. *L. delbruekii* and *Lactobacillus plantarum* showed higher storage viability. The viable cells remained at a maximum level within two weeks, but they decreased significantly after four weeks. Pomegranate juice has been proven to be an appropriate way to make a fermented probiotic beverage.

There has been a study into the effect of bacteria fermentation of lactic acid for sugars and organic acids, and the bio-metamorphosis for phenolic compounds (anthocyanins and ellagic acid) antioxidant activity of pomegranate juice were studied. *L. plantarum* and *L. acidophilus* were utilized as initiating probiotic organisms. The bacteria *L. plantarum* and *L. acidophilus* have been ready to increase within the juice, and their viable cells achieved 3.9 x 10^8 CFU/mL after 72 hours of fermenting. Fructose and glucose in the juice have been the important effect of consumed in couple starter microorganism cultures, while *L. plantarum* used high sugars consumed than *L. acidophilus*. The glucose deterioration value were more significant than the fructose. As the main acid found in the juice, the

concentration of citric acid were significantly reduced by both bacteria through the first 48 h of the process ($P < 0.05$).

Lactic acid were identified as the common, abundant acid metabolic (6.1 g/L) produce during the fermentation process, particularly by analyzing *L. plantarum*. LC/MS for various anthocyanins content showed that these compounds (except for pelargonidin 3-glucoside) were significantly deficient. In pomegranate juice after fermenting. DPPH has been demonstrated that fermenting of pomegranate juice using chosen probiotic garnish significantly increasing antioxidant activity [19].

1.2 Objective of the Thesis

This research aimed to determine the combined usage possibilities of ultraviolet (UV) and ultrasonic (US) processes in the non-thermal pasteurization of pomegranate juice. Finding the optimum point of co-pasteurization processes between UV rays and the US to pasteurize pomegranate juice, and then carry out the fermentation process of the pomegranate juice with *Lactobacilli's* bacteria to produce pomegranate juice with a high content of antioxidants and phenolic compounds comparison of unfermented juice as a result of bacteria fermentation and monitor the physiochemical change during the storage process for four weeks at 5 °C.

For this purpose, UV, US, and combined UV+US pasteurization of pomegranate juice were optimized using experimental design, 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, total anthocyanin, DPPH, HPLC TPC, and yeast and mold count and were used as quality parameters during all of the processes.

1.3 Hypothesis

The use of non-thermal pasteurization techniques such as ultraviolet radiation and ultrasound together to pasteurize pomegranate juice to preserve the biologically active compounds and the possibility of fermentation of pomegranate juice

obtained from the combination of the two methods together by *Lactobacillus plantarum* bacteria.

Therefore, it is assumed that the use of the two methods together in pasteurization enables us to preserve the nutritional value of pomegranate juice better than thermal pasteurization, and that the fermentation of pomegranate juice resulting from the use of non-thermal pasteurization with lactobacillus bacteria will enable us to improve the functional level, the nutritional properties of increasing the total phenolic content and antioxidant activates and sensory properties of pomegranate juice.

1.4 Food Preservation

Food preservation, while ensuring its safety and quality, has been a prime goal of food processors. The use of heat through thermal processing operations, including pasteurization, sterilization, drying, and evaporation, is common in the food industries to guarantee their microbiological safety. This traditional healing method relies practically on generating heat outside the product to be heated, combustion of fuels or an electric resistive heater, and its transference into the product through conduction and convection mechanisms.

Nevertheless, these processing types are also limited due to significant heat losses on the equipment and installations facades, decreased heat transfer power, and thermal damage caused by overheating due to the time taken to heat the food's thermal center. Inhibiting or eliminating microbes and ensuring food safety are essential tasks in modern food processing. Any food safety and quality shortage is considered a food with no value when manufactured [28]. When looking at the important point for inhibiting the micro-organism of food and juices, vegetables and fruits are essential to ensure their safety from microbial contamination and extend life expectancy of food and beverages because food and juices are still being an essential nutritional source for microbes activates and growth.

Conventional heat treatment process includes sterilization, pasteurization, and ultra-high temperature used in the pasteurization of juices to reduce harmful microorganisms to a safe standard that reduces health hazard and ensures food security. Nonetheless, this security standard generally reaches the cost of a

product, and it forms an unwanted oxidative taste degeneration, causes deficiency in nutritional value, and lacks vitamins and pigments. Although many improvement methods have been proposed to get optimum food safety without losing the nutritional value [29], the adverse impacts were decreased but not eliminated. Investigators and food manufacturers have been concerned with nonthermal technology to conserve the juice nutritional and quality [30]. Numerous no thermal technology for fruit and vegetable juices have been developed for this purpose, such as ultrasonically [31]–[35] high-pressure processing [36]–[40], thermosonication [41], [42], pulsed electric field [43]–[45], and irradiation or ultraviolet (UV) light processing [43], [46]–[52].

Despite the spread of modern industrial methods used in the pasteurization of juices and drinks, it were necessary to examine and compare all techniques and the suitability of producing good quality juices.

Consumers have had an increasing interest in fruit juices, which include bioactive compounds that provide positive health outcomes. These bioactive compounds include polyphenolic compounds with anticarcinogenic, antihypertensive, antimicrobial, and antioxidant properties[53], [54]. Fruit juices are categorized as essential components of health food and are highly encouraged and recommended for their regular intake. They include low-fat levels and the highest level of vitamin, mineral, and dietary fiber. They are essential resources of vitamin C and have the highest level antioxidant capacity (AOC) that helps to combat strain and prevent cardiovascular illness, atherosclerosis, hypertension, diabetes, and cancers [55]–[57]. Their intake has increased in recent years due to their natural flavor, nutritional value quality, and different health advantages [55].

To avoid the microbial contamination of unpasteurized juice and to ensure its safe consumption, the food manufacturers must pasteurize the liquid, and it is a step to reach the level of safety from microbial contamination [55], [58], [59]. Thermal pasteurization can inhibit and prevent the growth of microbes that cause spoilage of juices. Still, it have many negative effects on the nutrition of juice value and voluptuous properties [60]. Specifically, thermal pasteurization affects the color and anthocyanin content of the juices. Anthocyanin decomposes upon

thermal pasteurization, causing unwanted changes in color to brown or colorless, focusing on finding non-thermal methods for pasteurizing juices [61]. Therefore the demand for non-thermal treatments increased in eliminating microbes and preserving food quality [62]. Due to the increase in consumer requested for Products of high nutritional value and quality, among the developed non-heat food processing technologies, there are applications such as pulsed the electric field, high hydrostatic pressure (HHP), ultraviolet radiation (UV), ultrasonication (US), and ozone [63], [64]. These are new technologies that use low temperatures to reduce the microbial content of the food.

1.5 Food Manufacturers

Food manufacturers face many challenges, including growing consumer consciousness and demanding nutritious, more and safe food. Also, there is an enhanced advantage for vegetarian foods in place of animal products because of ideological, healthiness, environmental, and economic causes [65], [66]. The innovations entered within the food processing industry comprise the generation of health beneficial food products, i.e., foods that can advantageously influence particular human body functions, away sufficient nutritional impacts, leading to an enhanced health condition and health and illness loss hazard [67]. As such consider, probiotics as well as prebiotics an essential class of functional foods [39], [41],[43]. Likewise, incorporating the fruit into different food products increases its nutritional value and function quality, as the fruit is recognized as an essential source of bioactive molecules. For the above reasons, the market for fruit juices and drinks increases exponentially worldwide [67]. As a result, there is a type of research underway and focus on producing fermented juice, including fruit such as orange, apple, lemon, passion fruit, pomegranate, and etc. [71]. Specifically, pomegranate is an extremely nutritious fruit with three times the highest antioxidant activity than green tea [72]. Containing high levels of bioactive compounds like flavonoids and other phenol also has antioxidant, antimicrobial, and anti-mutagenic properties [73].

Functional foods are used around the world as targeted disease prevention agents [74]. While an outcome, beneficial health food possess received much care from

the food industries over the past few years [75], [76]. Global marketing for functional health food is on the rise. It constitutes one of the central regions of the invention concerning the food businesses [77], [78]. Generally, health beneficial food exerts advantageous health effects and comprises bioactivity and probiotic compounds [74]. Probiotics are microbes, and mostly bacteria additionally yeasts are frequently named useful or beneficial because they enhance the balance of enteric bacteria [79], [80].

Besides, several studies have indicated that consuming bio-promoting foods lowers cholesterol levels in the blood, strengthens the defense system, and prevents colon carcinoma [81]. Nevertheless, probiotics must exist in sufficient quantities in feed to provide their valuable possessions to the host [80]. Moreover, minimal viable cell concentrate of a probiotic feed product were measured to be around 10^6-10^7 CFU/mL throughout the time of usage [69]. In several research, it has been shown that the primary delivery compounds for probiotic bacteria are dairy products [82], [83]. Nevertheless, probiotic dairy produce has often unsuitable for consumption by specific communities of consumers because of higher lactose intolerance, allergies, lipid-containing, and vegetarian [84], [85]. As a consequence of increased consumer requests for alternate nondairy products for the supply of probiotic bacteria, scholarly and industry study has been triggering the evolution of innovativeness juice drink, and vegetable drink contains on probiotic bacteria [86], [87].

Todays, the market for fruit juices and drinks is showing a dynamic increase globally [88], [89]. Functional beverages made from fruit or vegetable drinks about built-in probiotic bacteria are an appealing alternative for those who never drink dairy produce [87]. Besides fruit juices, there have been reports be an appropriate new bearer undertakes a supply of probiotic bacteria because these are wealthy in minerals, vitamins, and antioxidant complex, that provide an acceptable growing substratum in equivalent with a healthy and robust appeal [71], [86]. Fruit drink fermentation through probiotic bacteria could rise cell vitality and improve the beverage produce's functional properties [71], [90]. As a result, various recent ongoing studies have been focused upon fermented juice drinks using different strains of probiotic organisms provide impressive results

[86], [90], [91]. Between the numerous fruits, pomegranate drink is highly regarded be early functional property because it has powerful antioxidant, anti-inflammatory, and anti-microbial properties, and it were earlier used for the sake of ferment by probiotic lactic acid bacteria that improve the juice health advantages [73], [92].

2

GENERAL INFORMATION

2.1 Pomegranate

Pomegranate (*Punica granatum* L.) is a popular agricultural yield grown in subtropics regions around the world. It is believed that its original homeland is central Asia, especially Iran and the regions around it, wherefrom it were spread in the world from these regions [93]. Todays, the Mediterranean countries and Asian regions are considered as the main centers for commercial pomegranate cultivation [94]. As every part of the pomegranate is rich in bioactive compounds, so its production is of great importance. Bioactive compounds offer numerous health advantages appropriate for several food pharmaceuticals, cosmetics [95], medication and nanotechnology applications [96] and can be deemed a superfood [97], [98].

The variety, location, and climatic conditions also influence pomegranate's chemical and biological properties and harvest ripeness [99]. The portion of the pomegranate fruit that is eaten consists of 29-54% of the grains or seeds, where it is approximately 80% juice and the rest 20% of seeds [100]. The portion of the pomegranate fruit that is eaten consists of 29-54% of the grains or seeds, where it is approximately 80% juice and the rest 20% of seeds, 1.5% fatty acids such as conjugated linoleic acid and linoleic acid, organic acids such as malic acid and ascorbic, citric, pectin, amino acids such as methionine, valine, and proline and another bioactive compound[100], [101].

The pomegranate fruit is often consumed either by eating its seeds naturally or by producing juice from the seeds by squeezing them, which has become very popular worldwide [102]. Reports indicated that pomegranate and pomegranate syrup have many functions: curative [103], preventing cancer and treating cancer [104], antimutagenic [105]. Action, hepatoprotective, neuroprotective and anti-inflammatory [106], antioxidant [99], and other health- raise effects [107]. Because there are antimicrobial properties in pomegranate, it may help to prevent

dental pathogen infection, bacteria *E. coli* O157: H7, and organisms that antibiotic-resistive likes MRSA. Another possibility applications contain brain ischemia in infants, and disease Alzheimer's, male infertility disease [108].

Recently, an extensive literature has formed about the issue of possible health advantage from PJ consumption [99]. Polyphenols are the major accountable for their strong antioxidant function, between the great numbering of phytochemicals contained in pomegranate fruits [109]. However, significant differences in the value and profile of phenolic complex and antioxidant ability were observed among the different PJs. For example, PJ pomegranate juice acquired from whole fruits differs distinctly from pomegranate juice made only from arils, marked by the prevalence of biomaterials anthocyanins, glutamines, hydroxy-cinnamic acids, dihydroflavonols, and hydroxybenzoic acids. Gallotannin and ellagitannins were the principal phenols extracted from whole fruit in pomegranate juice [110].

Aside from the variation between varieties, weather, growth environment, and the season and the country's geographical environment in which pomegranate is cultivated, in addition to many factors that determine the concentricity of phenolic compounds in the pomegranate fruit [99]. Verardo and Vegara (2013) it were recorded that flavonoids were 1.6-2.36% in PJ. The major flavonoids were anthocyanins (1.6−23.6%), found in pomegranate juice accountable for red color, while 16.4 to 65.8% of ellagitannins and phenolic acids are found [111], [112]. Thirteen different types of anthocyanins were found in pomegranate juice, in such a way as cyanidine 3,5-diglucoside and cyanidine 3-glucoside, delphinidine and pelargonidin were important anthocyanins identified. The following Table 2.1 shows the total anthocyanin content and total phenol in pomegranate fruit from 2009 to 2018.

Given that inner and outer factors, such as variety and environment, participate in the anthocyanin value. Anthocyanins, one of the flavonoids found in pomegranate juice, are unstable during the pasteurization process. As it oxidizes quickly under different treatment conditions of light, oxygen, and pH, and it is sensitive to different temperatures. Where the thermal pasteurization processes at 95 °C for 10 min of pomegranate juice had an effect on the stability of

anthocyanins and led to the appearance of brown pigments, and the red color were lost when compared to the unpasteurized sample, where the percentage of anthocyanin were lost after pasteurization, respectively 8–14% and 13–9% [113], where its instability is mostly due to its chemical composition [114].

Therefore, it is necessary to estimate changes in the number of anthocyanins in the juice during the pasteurization and storage period and its impact on the goodness of the juice. Where some acids that are found in pomegranate juice, such as hydroxybenzoic acids, such as gallic acid and ellagic acid, and hydroxy-cinnamic acids such as ferulic, caffeic, and chlorogenic, were specified, and they were the most important acids in pomegranate juice gallic acids there are about 12.42-88.51 mg/L in pomegranate juice and ellagic there are approximately 23.43-95.02 mg/L in pomegranate juice [115]. Ellagitannins and ellagic acid are the most important antioxidant compounds in pomegranate peels [116]. They can also been found in pomegranate juice produce by squeeze the full fruit.

Pomegranate, 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. There is much interest in pomegranate fruit juice (PJ) due to its high phenolic compound content, for example, anthocyanins, ellagic tannins, and catechins. Besides the many health benefits of bioactive compounds, anthocyanins are responsible for the bright red color, one of PJ's essential properties. The following table shows the nutritional value of pomegranate fruit Table2.2**.** This impacts one of the main concerns of consumers [61], [72]. Different pomegranate products are given in Figure 2.1.

Table 2. 1 Anthocyanin and total phenolic contents of pomegranate juices from different origins

Number of items	Pomegranate growing country	Total phenols (mg GAE/J_{uice})	References
20	Iran	2960-9850	[117]
9	Tunisia	458-3299	[118]
76	Turkey	1080–9449	[119]
10	Morocco	410.1-834.3	[120]
17	Turkey, Israel, Spain, Tunisia, Italy, Iran	580-2551.3	[111]
6	India	876.2-1536.2	[121]
6	Spain	88.5-173.2	[122]
19	Spain	90-145	[123]
16	Sicilia and Spain	857.0-3097.7	[124]
18	Morocco	1385-9476	[115]
7	Italy	870-1930	[125]
Number of items	**Country of agriculture**	**Total anthocyanins (mg GAE/J_{uice})**	**References**
8	Iran	2380-9300	[126]
7	Turkey	81-369	[127]
8	Chile	168-1328	[128]
9	Tunisia	11-178	[118]
15	Spain	34-1075	[129]
16	Sicilia and Spain	857.0-3097.7	[124]

Figure 2. 1 Different pomegranate products

Table 2. 2 Nutritional value of pomegranate juice according to the united states department of agriculture (USDA)

Nutritional value	Unit	Value per 100 g
Water	g	77.93
Energy	kcal	83
Protein	g	1.67
Gross lipid	g	1.17
Total carbohydrate	g	18.70
Total Fiber, dietary	g	4.0
Total Sugars	g	13.67
Zinc, Zn	mg	0.35
Potassium, K	mg	236
Magnesium, Mg	mg	12
Sodium, Na	mg	3
Iron, Fe	mg	0.30
Phosphorus, P	mg	36
Calcium, Ca	mg	10
Vitamins		
Total ascorbic acid ,V-C,	mg	10.2
Riboflavin	mg	0.053
Niacin	mg	0.293
Thiamin	mg	0.067
Vitamin B-6	mg	0.075
Vitamin K (phylloquinone)	microgram	16.4
Folate, DFE	microgram	38
Vitamin A, RAE	microgram	0
Vitamin A, IU	IU	0
Vitamin B-12	microgram	0.00
V (D2 and D3)	microgram	0.0
V-D	IU	0
V-E (alpha-tocopherol)	mg	0.60
Lipid		
Total monounsaturated fatty acids	g	0.093
total Saturated fat	g	0.120
Total polyunsaturated fatty acids	g	0.079
Total trans Fatty acids,	g	0.000
Cholesterol	mg	0
Other		
Caffeine	mg	0

2.2 The Chemical Composition of Pomegranate Fruit

The improved antioxidant function of pomegranate products has contributed to health studies of multiple chronic diseases associated with oxidative stress. In several trials, pomegranate fruit and juices are antioxidant better from another food with healthy antioxidant levels, such as green tea and wine, using various measures [72], [130]–[133]. Hydrolyzable tannins and anthocyanins, especially ellagitannins, release ellagic acid when hydrolyzed are the key groups of phytochemical elements known for pomegranate fruit Figure 2. 2 [134] .

Where pomegranate juice were for most ellagitannins in 29-line found on ellagic acid, gallagic acid, punicalin, and punicalagin inhomogeneous pomegranate juices over two seasons of growth [135]. The study showed that the quantification of antioxidants in pomegranate juice were estimated by the equivalent capacity of the antioxidants equivalent to trolox, and the antioxidant capacity equivalent to ascorbic acid is primarily due to the concentricity of hydrolyzed tannins with very little anthocyanin contribution in vitro [72]. Antioxidant production were also considerably associated with total polyphenols in whole fruit pomegranate homogenates, but not with gross anthocyanin value[135].

Although anthocyanins have been connected with health impacts, including preventing obesity, diabetes, and cardiovascular disease [136], must be not neglecting the bioactive ingredients of pomegranate. Cultivars of cyanidine 3,5-diglocoside, delphinidin 3,5-diglucoside, cyanidin 3-glucoside, pelargonidine 3,5-diglucoside, pelargonidine3,5-glucoside were the significant anthocyanins in pomegranate juices in many Iranians [126], [137]. Extracts of pomegranate peel were showed to have more significant antioxidant activity from the juice [138] or seed extracts [139] and were active at preventing lipid peroxidation [138], [139] Ex vivo LDL oxidation at amounts between 50 ppm and 100 ppm of peel extract. The pomegranate peel extract has also proven an antioxidant more powerful than ascorbic acid vitamin C or turmeric, two food-derived compounds known as antioxidants [138]. Simultaneously, pomegranate seeds have the lowest phenolic content than the antioxidant capacity in vitro [139]. The oils derived from them include additional elements that could provide positive effects on health.

The seed oil has a high phytosterol content and a special fatty acid profile, including punic acid Figure 2. 3 [134], an isomer of linolenic acid conjugated [140]. Punic acid were 70% to 76% of pomegranate seed oil across 15 Turkish pomegranate cultivars. The oil balance consisted of α-eleostearic, palmitic, behenic acids, linoleic,gadoleic arachidic, oleic, stearic, behenic acids catalpic, and β-eleostearic [141].

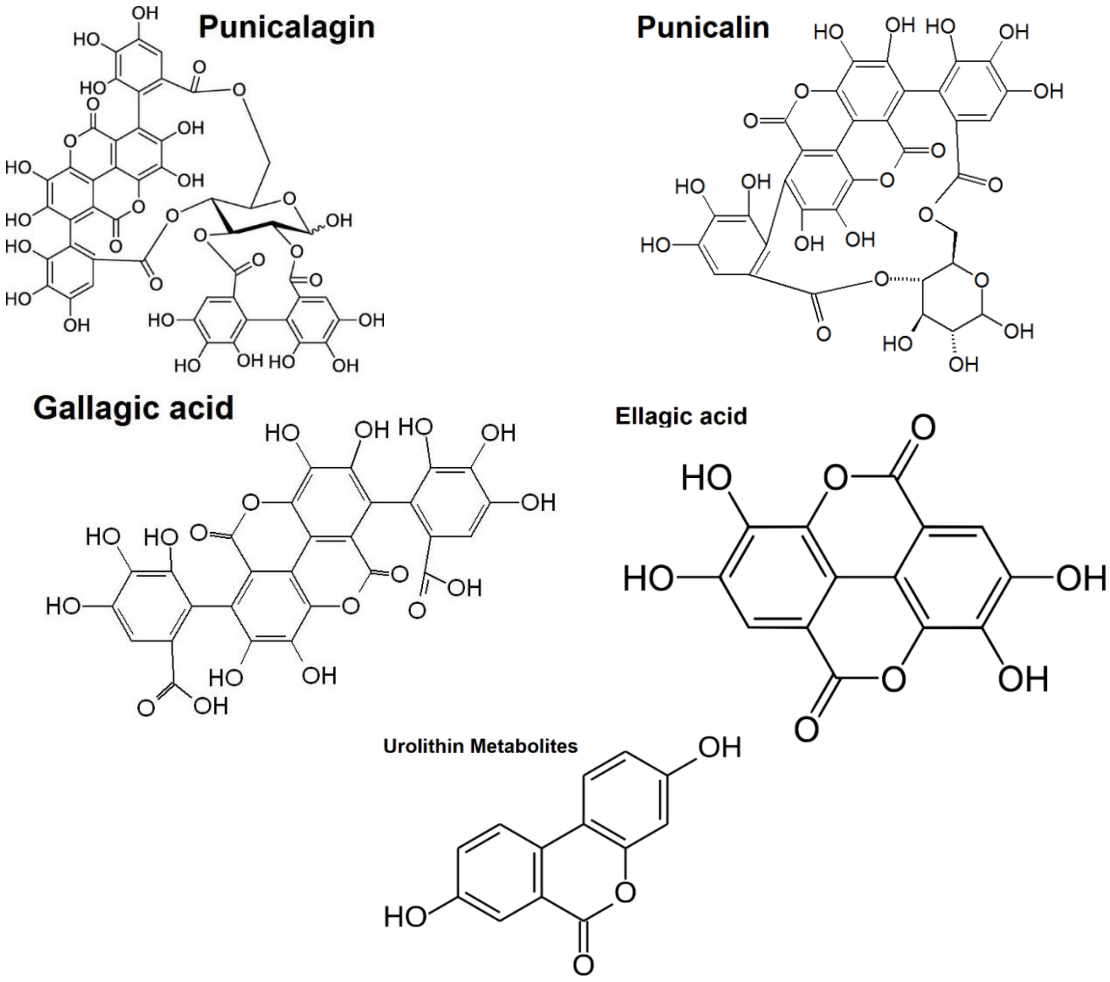


Figure 2. 2 The chemical composition of pomegranate

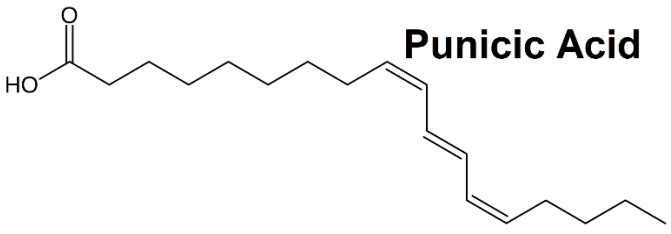


Figure 2. 3 Chemical structure of punicic acid, a joint with linolenic acid in pomegranate juice

2.3 Changes in the Quality of Pomegranate Juice because of Treatment

The fruit beverage market has increased steadily for years because the consumer attention to drinks and nutritious foods has increased [102], [142]. The effects of the health interest of pomegranate have improved fruit consumption, whether fresh or frozen. Pomegranate juice can be obtained through several methods, pressing the pomegranate seeds or pressing the pomegranate fruit in its entirety, or by pressing the half-cut pomegranate fruit [115], [143]. If you pressure squeeze pomegranate juice, there is a bitter taste due to the tannin content of the peeling, to altogether avoid the bitter taste resulting from pressing the pomegranate fruit ultimately, which is that must be only press the pomegranate seeds even though the bitter taste contains more antioxidants than pure juice [72]. The different treatment stages that pomegranate juice is subjected to in factories, from thermal pasteurization, filtration, and enzymatic treatment, may significantly affect the physical and chemical properties and antioxidants of pomegranate juice.

2.3.1 The Effect of Heat Treatments on Juice in General and on the Pomegranate Juice in Particular

The thermal process intensity is usually applied however, it deteriorates the nutritional quality of fruit juices and changes their physicochemical and sensorial properties [58], [144]–[146]. These restrictions have aroused research and treatment evolution with minimum effect on the properties aforementioned of fruit juices [147]. Pomegranate juice serious concern is microbial contamination with acid-resistant yeasts or mold, which leads to degradation of nutritious and sensory property like color, smell, and flavor [148]. The percentage of microbial contamination depends on the process of manufacturing PJ, fruit type, harvest time, and processing conditions[149]. The expectation of consumers for healthy food is increasing with the improvement of technology. Now a days, to improve the shelf life of food, microorganisms and enzymes are inhibited by chemical, thermal, or combined processes [150]. The conventional thermal pasteurization methods use the conditions that are 63 °C at 30 min, 72 °C at 15 sec, or 90 °C at 5 sec [60].

Pasteurization is primarily needed by inactivation of the micro-organisms and enzymes endogenous to prolong the shelf life. According to the FDA, the 5-log decrease of *Salmonella* spp., *Listeria monocytogenes*, *E.coli,* and *cryptosporidium partum* oocysts of requires 71.7 °C for 15 seconds in fruit juice with a pH of less than 4.0 [146]. The anthocyanins' degradation leads to a change in the juice color due to the formation of colorless polymeric dyes during processing. Thar are two types of thermal pasteurization, which is either slow pasteurization at a temperature of 63 for not less than 30 min or pasteurization rapid at 72 °C for 15 seconds [151]. Through pomegranate juices obtained from many varieties or by obtaining a mixture of lemon juice and pomegranate juice, the changes that occur to the color and the changes that occur to the phenolic compounds and also the changes that occur to the vitamin C when heat pasteurization using a temperature of 65 for 30 s or use a temperature of 90 for 5 s are less clear [152]. In any case, both pasteurization process may have adverse impact on the nutritional value and pomegranate juice physicochemical properties Table 2.3.

Table 2. 3 Effect thermal pasteurization on the overall quality of pomegranate juice

Sample	The heat used for pasteurization and microbial, physical, and chemical analysis of juice	References
Cloudy pomegranate juice **Cloudy clarified juice**	60 °C for 30 s, 95 °C for 5 s. Store at 25, 5 °C for forty-five days to a hundred and twenty days. Both pasteurization processes were able to inhibit microbial growth for 120 days when stored at 5 °C. The red color loss minimal in pomegranate juice stored at 6 °C compared to samples stored at 25 °C, the red color loss faster. The Browning indexes increased at an unacceptable value after 7 days of storing at 25 °C for pomegranate juice. While pomegranate juice stored at 5 °C were of little less browning.	[112]
Cloudy pomegranate juice obtained from the seeds and purified by a nylon mesh	The temperature used for pasteurization is 80 °C for thirty seconds. Fresh pomegranate juice stored at 5 °C. The pasteurize pomegranate juice were storage at room temperature for four weeks. Thermal pasteurization inhibited the microorganism in pomegranate juice during storage. Thermal pasteurization led to increasing the amount of ellagic acid and gallic acid in pomegranate juice.	[153]
Cloudy juice contained 20 g/L pulp	65 °C for 30 s, 65 °C for 60 s, 80 °C for 30 s, 80 °C for 60 s, 90 °C for 30 s, 90 °C for 60 s. At 65°C were able to reduce by 4 log approx. the number of Aerobic Plate Count which may not be able to grow in storage under cooling conditions. At 90 °C, were inhibit all Aerobic Plate Count. The anthocyanin content was improved after heat treatment by 10% when LTP pasteurized juice, with moderate and high pasteurization, anthocyanins were enhanced by 56% in the juice. Notice an increase in a* value upon completion of pasteurization. Thermal pasteurization did not affect the total phenol content of the treated Pomegranate juice in all samples.	[152]

Table 2. 3 Effect thermal pasteurization on the quality of pomegranate juice (Continued)

Cloudy PJ from arils	Pasteurization were done in a small scale laboratory tubular pasteurization at 80 °C for 2min growing of ferulic acid, ellagic acid, punicalagin 1, and catechin in PJ after juice pasteurization	[114]
PJ-A Whole All steam at the highest pressure PJ-B full fruit not steamed, Maximum steam pressure PJ-C Juice from the seeds and monocarp clarified	The thermal pasteurization process done at 60, 70, and 80 °C, and the treatment time 15, 30, 60, 90, 120, 180, and 300 min. It has been observed that the temperature of 60 °C and 70 °C is not sufficient for the pomegranate juice to be pasteurized. The temperature is 80 °C and 90 °C the pasteurization time is 31.2 and 2.4 minutes, respectively. The anthocyanin content in pomegranate juice not affected by the content of the phenolic compounds and low/high molecular pomegranate matrix components organic acids and sugars. The antioxidant capacity and total phenol content were not significantly altered by the different temperatures when pasteurized.	[110]
Cloudy PJ from arils centrifuged 2 min at 10 000 rpm at -4 °C	Pasteurization the juice by thermostatic water bath at 85 °C for five min 4 °C for ten days. In pasteurized PJ, debasement of individual and total anthocyanins were about 42.8 ± 0.5% after storage. The reducing ratio of anthocyanins in Pasteurized juice throughout store were lower section stored fresh PJ due to thermo deactivation of polyphenol oxidase (PPO).	[137]
Comparison between filtered and unfiltered juice from pomegranate seeds and entire fruits	Pasteurization of pomegranate juice in a water bath a temperature of 95 °C for ten min. Anthocyanin value for unresolved pomegranate jui from seed and the full fruit were nearly similar, whereas clarification of juice caused anthocyanin to decrease by 4% from juice obtain by arils and 19% from juice obtained by whole fruit.After pasteurization process, the content of anthocyanins decreased by percentage in pomegranate juice from the seeds an 8-14% when compared to pomegranat juice from the whole fruit 9-13%. After pasteurizatic the polymeric color increased in both samples of th juices.	[113]

2.4 Non-thermal Treatment for Juice Preservation

Novel technology that give the minimal impact of food manufacture and maintenance on function characteristic whilst preserving goodness and safety are drawing lots of interest [154]. These technologies' main challenge is overcoming all the harmful effects of conventional heat treatment although preserved all of their benefits [155]. Some of the non-thermal methods with the potential to replace thermal processing of foods include ultrasonic and ultraviolet treatments [156], [157].

2.4.1 Ultraviolet Treatment UV-C

Between the non-thermal technologies for the conservation of fruit juices, ultraviolet radiation (UV) has been shown to improve life expectancy and more comfortable use while decreasing costs of compared with different alternate. This technique can decrease bacterial numbers in liquid foods and drinks outwardly negatively influence goodness in terms of physicochemical criterion, sensitive characteristics, and bioactive composites [48], [158]. Ultraviolet (UV) rays are a small portion of the electromagnetic spectrum. The wavelength of UV irradiation process extends from 100 to 400 nm, and be classified UV-A (320–400 nm), UV-B (280–320), UV-C (200–280 nm), and the vacuum UV range (100–200 nm) (Figure 2. 4) presents the position of the UV area in the electromagnetic spectrum [159].

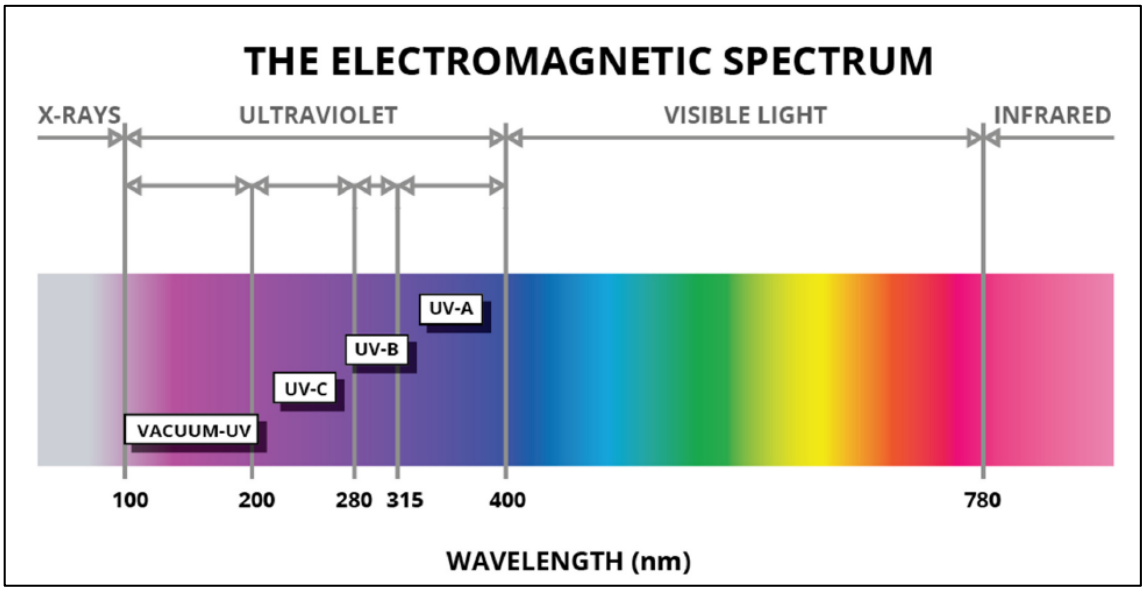


Figure 2. 4 UV wavelengths

2.4.1.1 UV Apparatus

Many UV reactors are designed for use in the pasteurization process of juices and drinks. The main ones are turbulent flow devices, laminar flow devices, and column flow reactors. As in Figure 2. 4, which contains two overlapping cylinders, as these cylinders rotate on their axes and have low rotational speeds, it is known as the Taylor - Quiet flow. The outer surface of the cylinders is made of quartz glass. Aluminum reflectors are located on quartz glass. Where vortices are generated as a result of operating the reactor, and these are called vortices [160].

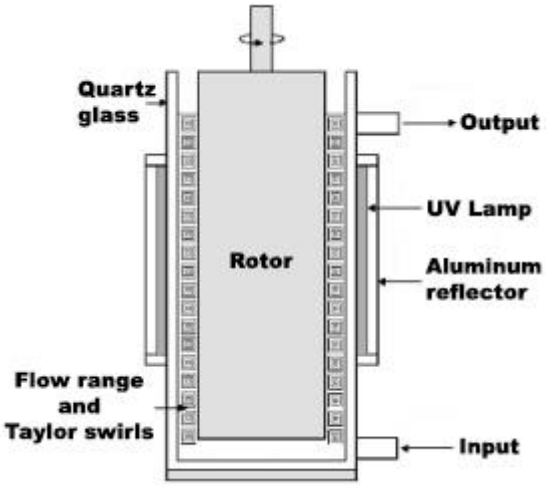


Figure 2. 5 Laminar flow UV reactor

The purpose of the turbulent flow reactor design shown in Figure 2. 5 were to increase turbulence within the reactor and with a uniform flow rate and high flow velocity. Thus, better results are expected by exposing each product's drop to UV rays [160].

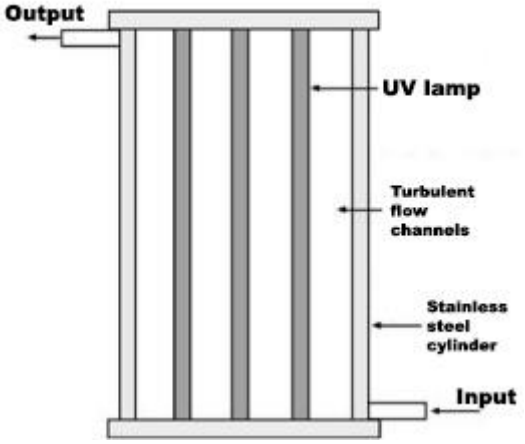


Figure 2. 6 Turbulent-flow UV reactor

The reactor Figure 2. 6 contains coiled tubes in a spiral and UV lamps, as there are reflectors inside and outside the tube where the device's outer housing is made of a stainless steel cylinder. A second vortex is formed by the coiled telephone tubes, and this is called the DIN effect [160].

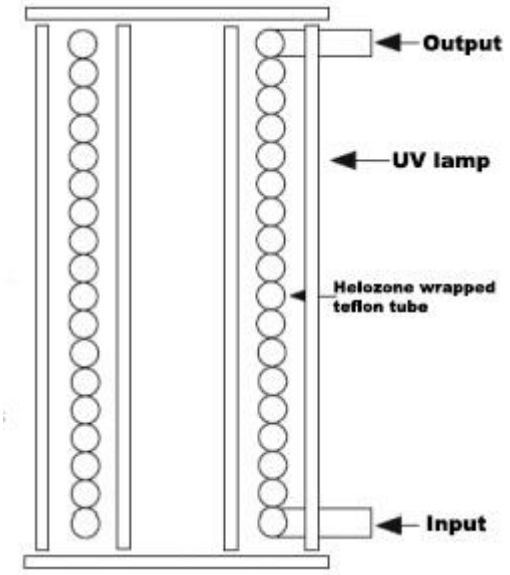


Figure 2. 7 Dean-flow UV reactor

2.4.1.2 Breakthrough of UV-C

Ultraviolet penetration into liquids depends on the fluid's ability to absorb UV rays, the Brix value, and the liquids' suspended matter content. The penetration rate of ultraviolet rays decreases when drinks contain a high Brix value and when they also contain a large proportion of suspended substances, which will be counterproductive to the effect of ultraviolet rays on microbes [159], [161]. Likewise, color components and organic compounds present in juices and drinks affect the absorption of ultraviolet rays, and that when compared to water, it were noticed that *Escherichia coli* K12 [162]. A study proved that increasing the content of suspended matter in apple juice reduced ultraviolet radiation efficiency from eliminating bacteria [163]. Also, the penetration rate of UV fluids depends on the design of the device[160].

2.4.1.3 UV Mechanism

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 [161]. UV lights are mostly adsorbed by microorganism DNA and block DNA transcription and bond translation in the same DNA strand via adjacent pyrimidine bases [164].

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, provides scientific parameters for juices' pasteurization. This were a 5 log reduction of the most resistant microorganism threatening to public health [50]. UV radiation were proven to be a safe method used in the pasteurization of juices by the US food and drug administration (FDA), [160] and the united states department of agriculture (USDA). The effectiveness of UV light in apple, grape, cranberry, grape juice, and orange juice has also been demonstrated by various studies [164], [165]

In specific, Ultraviolet radiation type C is bactericidal at 254 nm and is utilized as a antiseptic process to prevent or deactivate food-borne micro-organism in fluid food produce. It were widely used when the US food and drug administration

approved it as a substitute for pasteurization cold pasteurization in the year 2000 (US FDA, 2000) [166]. DNA in the microbial cells soak up light through the UV-C process of food and forms DNA photoproducts, which is the main inactivation impact of UV. The maximum significant production is the pyrimidine dimer made between neighboring pyrimidine molecules on the same DNA strand. These molecules can prevent DNA translation and transcription, generate in cell death, which inhibits their reproductive treatment and outcomes in microbial inactivation [167] Figure 2. 7.

Damage from ultraviolet light therapy leads to dimethylamine that causes folds in the DNA strands and disrupt chromosome proliferation before cell division. Hence, gene transcription cannot take place. If this thymine alteration is seen in genes that perform vital functions, it is fatal because DNA replication is inhibited. As a result, it becomes difficult for the microorganism to repair itself (Özkütük, N., (2005)

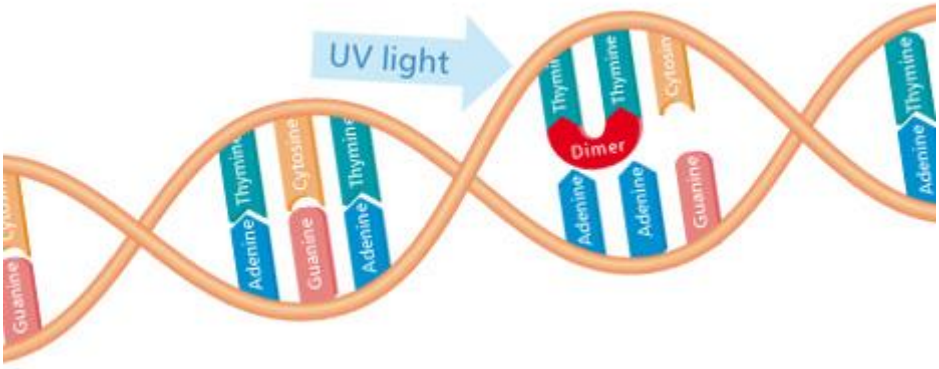


Figure 2. 8 Mechanism of UV treatment affecting DNA

Although UV-C radiation as a non-thermal technique is common use to preserve fruit drink, few studies have used ultraviolet light in the pasteurization of pomegranate juice, including [2], clarified pomegranate juice obtained from arils were be treated by UV-use lamp doses 254 nm, 28 W UV- C output from 125 to 62.4 J/mL UV doses.

The pomegranate juices, pasteurized by ultraviolet radiation, maintained the quality of the pomegranate juice. They preserved a large percentage of the juice anthocyanin content compared to the thermal pasteurization at a temperature of 90 °C for two minutes. Where a decrease in anthocyanin content were observed were between 8.1% and 16.3%. Reducing the aerobic platelet count, yeast, mold, and *Escherichia coli* count (ATCC25922) as an alternative micro-organism to *E. coli* O157: H7 in PJ outcomes in logarithmic reductions as follows: 1.8, 1.45, and 6.15 CFU/mL log respectively. Supports the results obtained when using UV-C light for antiseptic and extending pomegranate juice service life over cold storage 4-10 °C without negative effects on the physical, chemical, and nutritional value.

2.4.2 Ultrasound for Pomegranate Juice Processing

Ultrasound (US) is a non-thermal technique used to preserve foods where the thermal coefficients affect food products' nutritional value, 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 [10], [169]. The ultrasound technology has been used for character enhancement and inactivation of microorganisms in orange juice [170]–[172], strawberry juice [173], blueberry juice [174], tomato juice [150], [175], cactus pear juice [176], apple juice [41], [52], [172], [177]–[179], lime juice [180], pear juice [181], carrot juice [42], and a blend of different fruit juices [34], [35].

Ultrasound (US) treatments has been used effectively for various purposes in the food industry, including manufacturing, storage, and extraction. Ultrasound is a cyclic pressure wave with a frequency higher than the standard human hearing range (20 kHz-10 MHz) [32]. Ultrasound causes specific effects (e.g., physical, chemical) when moving through a medium, usually deemed non-thermal. The efficiency of ultrasound purposes as an option to conventional pasteurization is based on improving temperature and mass transfers. These are attributable to decreased heat and mass transfer resistor at the interface and are often related to cavitation activity[182]. To use ultrasound as a possible option for pasteurization

in fruit juice products, it is essential to consider the sub-lethal injury caused on microbial cells and their ability to freshen when status be more helpful [183].

2.4.2.1 Mechanism

The ultrasound power mechanism produces strong ripples from cavitation in liquid solutions according to the juice characteristics, air presence, and ultrasonic system acoustic power. The 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, contributing to microbial inactivation [184], [185].

The mechanism of the inactivation of microorganisms primarily depends on physical and chemical factor. The physical effects that occurred throughout treating in the US can be described as the increase in perturbation throughout the medium. At lower frequencies (<100 kHz), turbulence occurs predominantly from a propagating shock wave and high-energy bubble collapse. This can reason the polymer chains and cell walls to break. Chemical effects are most prevalent at medium frequencies (200-500 kHz), where the number of active bubbles generated be higher. At larger frequency of the audio stream (> 1 MHz), the predominant effects are related to cavitation and then physical and chemical effects [186]. However, the type of microorganism is an essential factor in disrupting ultrasound treatment therapy Figure 2. 9. For successful microbial inactivation, several factors should be considered, such as shape (cocci or Bacilli) and diameter of microorganisms [187], the cell size or surface area (large or small), gram positivity or negativity (cell wall thickness), and cell sensitivity or ability to recover from treatment [188].

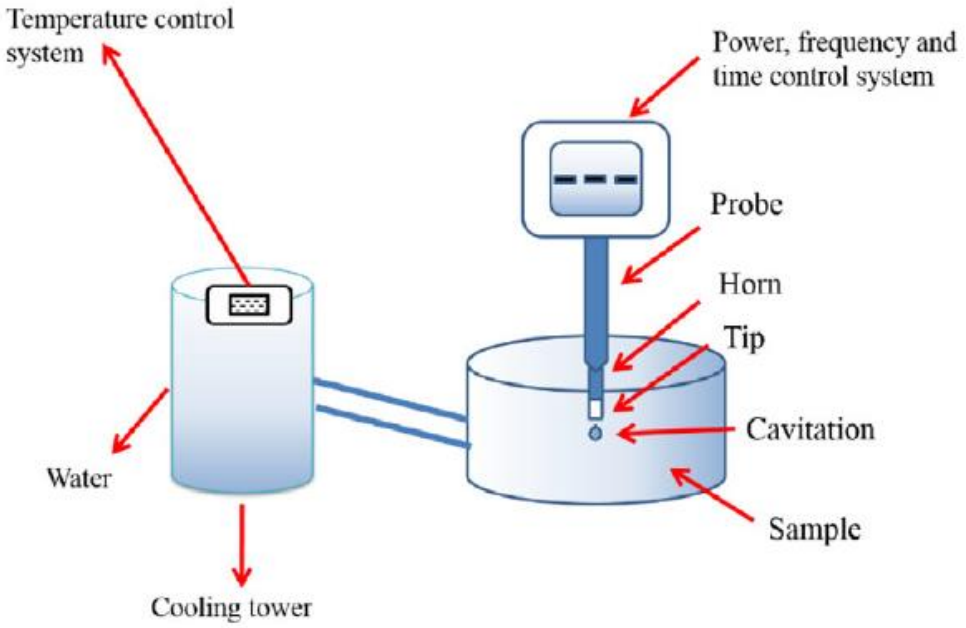


Figure 2. 9 Schematic diagram of sonication systems

3
MATERIAL AND METHOD

3.1 Materials

Fresh pomegranate (*Punica granatum*) fruits were purchased from a domestic market in Istanbul, Turkey. The pomegranate fruits were kept in the Yildiz Technical University's cold storage food engineering laboratory at +5 °C until being subject to the procedures. All chemicals were purchased from (Merck, Darmstadt, Germany).

3.2 Preparation of Pomegranate Juice (PJ)

Healthy pomegranate fruits were selected and washed. The seeds were separated manually, and the fruit were then squeezed in a juicer (King P–1120 Vitamix Juicer 400W, Turkey). Figure 3. 1 shows a diagram showing the operations to extract and pasteurize pomegranate. In the conventional pasteurization process, the temperature were set at 72 °C in the water bath (Daihan, WUC-D10H, South Korea), and the juice were allowed to be pasteurized for 15 sec.

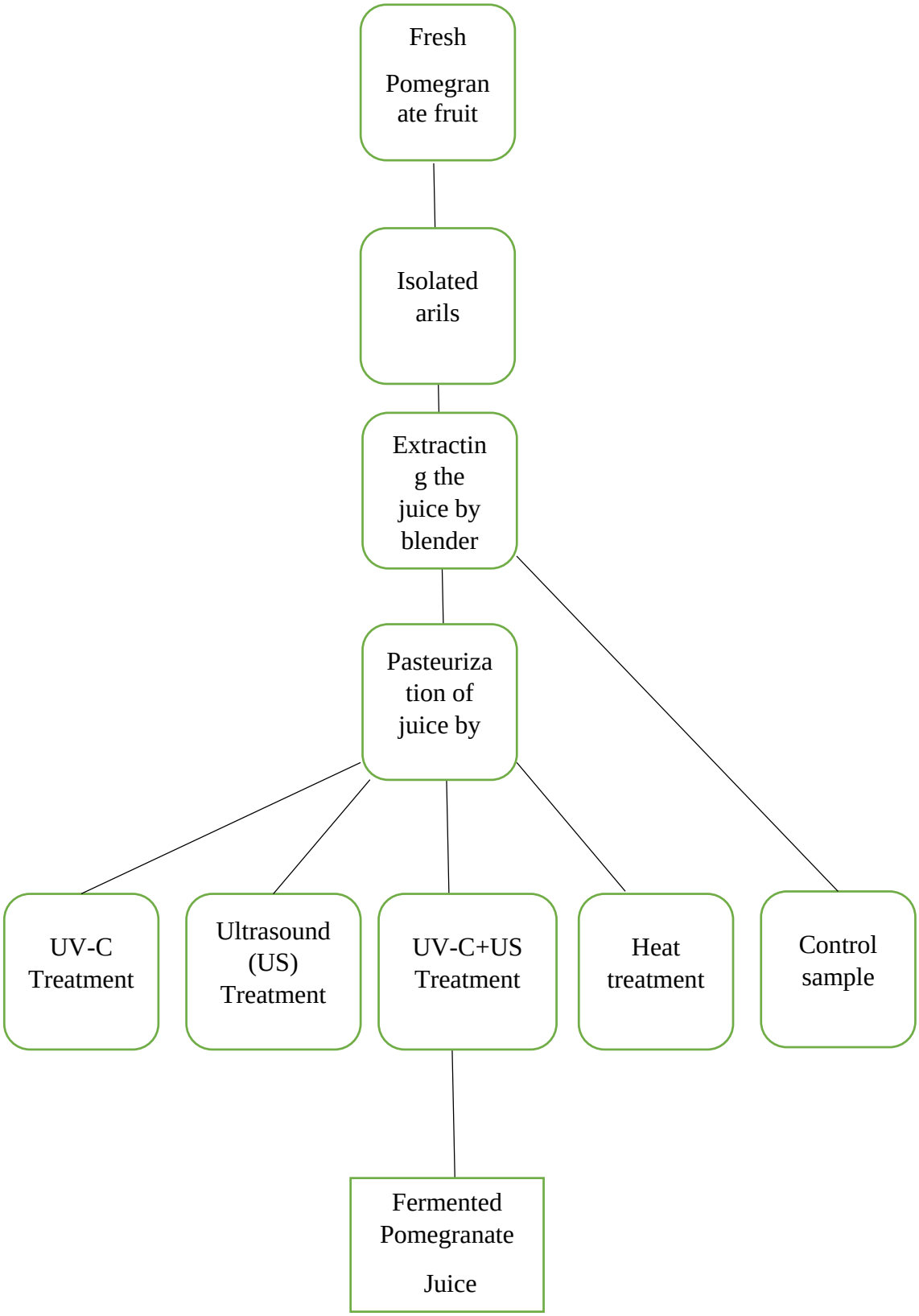


Figure 3. 1 Shows a diagram of operations to extract and pasteurized pomegranate

3.3 Fermentation of Final Beverage by *Lactobacillus plantarum*

Lactobacillus plantarum probiotic strain were grown beneath anaerobic status at the 37 °C for forty-eight hours in MRS broth. Wet biomass crop through centrifuge at 5000 revolutions per minute for ten minute at 25 °C. Optimum points obtained from treatment by UV-C & US 100 mL were took from the juice and transferred within 250 mL flask, 1 g of collected wet weight *Lactobacillus plantarum* were adding to 100 mL of pomegranate drink in order to fermented in 30 °C within twenty-four hours. After 24 hours, the cell viability were determined at 9.64 log CFU/mL of juice. Then, the fermented pomegranate were preserved within 5 °C into twenty-eight days. All cell counts were declared value as a log of average colony forming units CFU/mL of pomegranate drink. All outcomes are submitted as means of three recurrent plus standard deviation.

3.4 Ultraviolet Treatment

Pomegranate juice samples were pasteurized with a CiderSure 3500 UV pasteurization device (Figure 3.2, 3.3, 3.4) [189] under different process conditions (temperatures, flow rates, UV doses). The CiderSure 3500 device has been approved by the FDA. *Salmonella* and *Cryptosporidium* strains and *Escherichia coli* strains [187]. The device operating system is shown in the following diagram. UV exposed PJ samples were aseptically filled into sterile glass bottles (100 mL) and stored at +5 °C for 28 days.

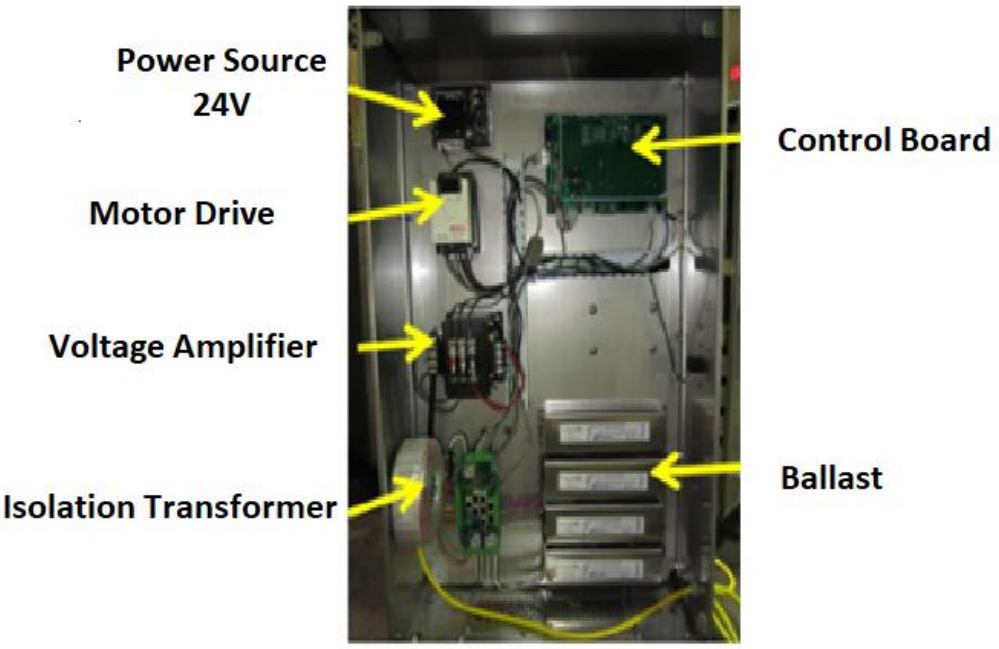


Figure 3. 2 Front internal view of the 3500 CiderSure devices

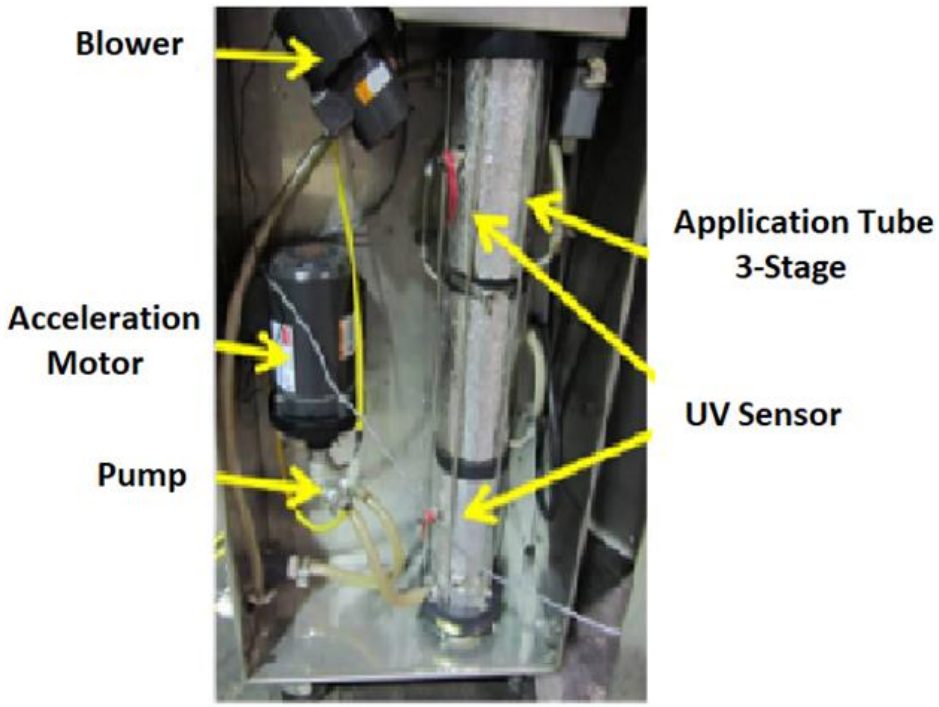


Figure 3. 3 Rear internal view of the 3500 CiderSure devices

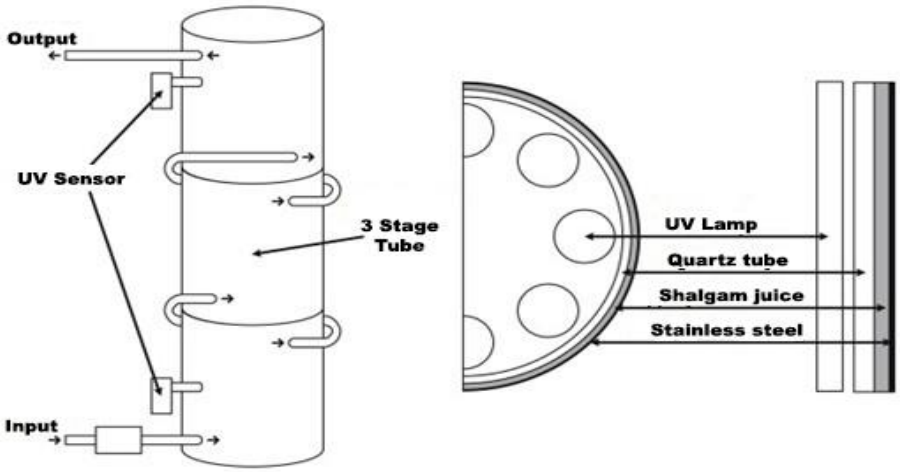
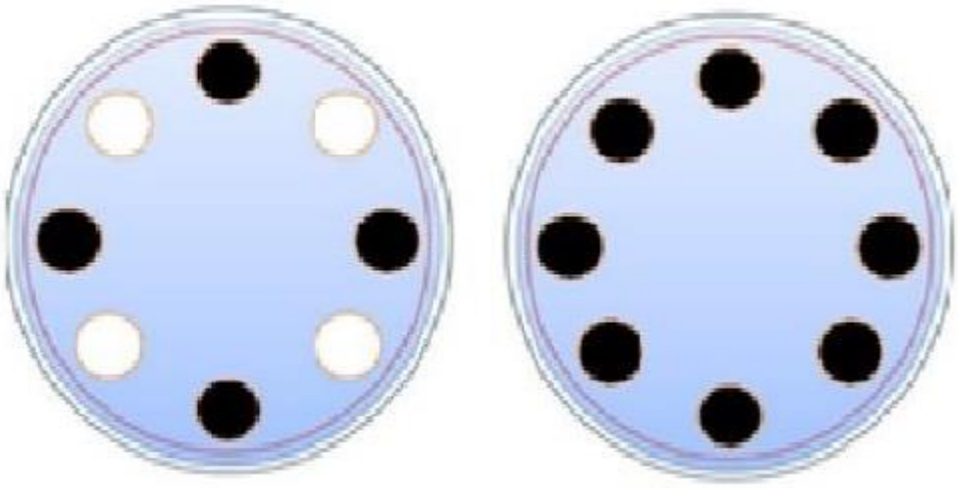


Figure 3. 4 Schematic representation of CiderSure 3500 UV device

3.4.1 Design of Treatment Conditions

At the beginning of the design, 12 pomegranate juice samples were treated with ultraviolet rays under different treatments. First, the speed of juice flow inside the device at two different speeds (1.5, 3.5 L/min), and three different (40, 50, 60 °C) temperatures were used. UV lambs were used at two different levels (1 lamp and 2 lamps) as shown in Table 3.1. The UV lamps' design in the study is shown in Figure 3. 5. The control sample were not subjected to any pasteurization process.



DOSE I DOSE II

Figure 3. 5 The design of UV lamps

The device must be cleaned before using it, among the things that must be done when cleaning the device. The warm water (40 °C) must be passed continuously for 5 minutes, after which the distilled water (~15 °C) should be passed for 10 minutes. Then the HCl solution (0.5% v: v) solution should be passed for 10 minutes, and after this solution, must be pass distilled water (~15 °C) at room temperature for 10 minutes. They were then passed the NaOH solution (0.1% w:v) for 10 min and then rinse the device with distilled water (~15 °C) for distilled water. The last stage passed the NaCl solution (6% w:v) for 10 min and finally cleaned the device from this solution with distilled water for (~15 °C) min.

Table 3. 1 UV process conditions used for pomegranate juice pasteurization

Run Order	Temperature (°C)	Flow rate (L/min)	UV lamp series	UV Dose (mW/cm2)
1	50	1.5	1 LAMP	5.1
2	60	1.5	1 LAMP	5.1
3	50	3.5	2 LAMP	10.1
4	40	1.5	1 LAMP	5.1
5	40	3.5	2 LAMP	10.1
6	50	3.5	1 LAMP	5.1
7	50	1.5	2 LAMP	10.1
8	60	1.5	2 LAMP	10.1
9	40	3.5	1 LAMP	5.1
10	40	1.5	2 LAMP	10.1
11	60	3.5	1 LAMP	5.1
12	60	3.5	2 LAMP	10.1

3.5 Ultrasound Treatment

The acoustic wave system used in juices' pasteurization is one of the widespread non-thermal techniques and has several beneficial properties for the pasteurization of juices without using high temperatures and extending the juice shelf life [188]. At the beginning of the design, the 18 pomegranate juice treated with ultrasound. PJ with the amount of 250 mL were put into a vessel glass with a cooling cylindrical jacket. It were 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 were 25 °C, and the flow of water were 2.1 L/min. Considering the experimental design, ultrasound power (50%, 75%, and 100%; 165, 200, 295 W, respectively) were applied to the PJ. The temperature were adjusted to 40, 50, and 60 °C. The increase in the power of sonication increased the temperature of the sample, as well. Since it were challenging to decrease/increase the temperature using 50% (165 W), 75% (200 W), and 100% (295 W) ultrasound power. The temperature of the sample were 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, as explained in Table 3.2. Optimum conditions for US treatment were decided considering TPC and yeast and mold counts. Figure 3. 6 explains the ultrasound device and all sample were stored at +5 °C for 28 days.

Figure 3. 6 Ultrasound device

Table 3. 2 US process conditions used for pomegranate juice pasteurization

Run Order	Temperature (°C)	Time for Treatment	Device is on or off	Power for ultrasound	Watt
1	50	10	ON	75%	200
2	40	10	OFF	0	0
3	50	15	ON	75%	200
4	40	15	ON	50%	165
5	60	5	ON	100%	295
6	60	10	ON	100%	295
7	60	5	OFF	0	0
8	50	5	OFF	0	0
9	40	15	OFF	0	0
10	40	5	OFF	0	0
11	60	15	ON	100%	295
12	60	10	OFF	0	0
13	60	15	OFF	0	0
14	40	10	ON	50%	165
15	40	5	ON	50%	165
16	50	15	OFF	0	0
17	50	5	ON	75%	200
18	50	10	OFF	0	0

3.6 UV and Ultrasound Treatment

After determining optimum conditions for UV and US processes, these two methods were used in combination. Briefly, PJ 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 were used for further analysis, as explained in Table 3.3.

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 analyses cannot be performed one days, treated, PJ stored at +5 °C and stored for 28 days.

Table 3. 3 UV and US process conditions used for pomegranate juice pasteurization

Run Order	Temperature (°C)	Time for Treatment	Flow rate (L/min)	UV lamp series	UV Dose (mW/cm2)	Power	Watt
1	40	5	3.5	1 LAMP	5.1	50%	165
2	40	10	3.5	1 LAMP	5.1	50%	165
3	40	15	3.5	1 LAMP	5.1	50%	165
4	50	5	3.5	1 LAMP	5.1	75%	200
5	50	10	3.5	1 LAMP	5.1	75%	200
6	50	15	3.5	1 LAMP	5.1	75%	200
7	60	5	3.5	1 LAMP	5.1	100%	295
8	60	10	3.5	1 LAMP	5.1	100%	295
9	60	15	3.5	1 LAMP	5.1	100%	295

3.7 Thermal Pasteurization of Pomegranate Juices

In the conventional pasteurization process, the temperature was set at 72 °C in the waterbath (Daihan, WUC-D10H, Seoul, South Korea). The juice allowed to be pasteurized at this temperature for 15 sec. Conventionally pasteurized PJ were used as a control.

3.8 Pomegranate Juice Storage

UV treated, US treated, UV+US treated, and conventional and control and fermented 200 mL pomegranate juice samples were stored at 5 °C for 28 days (Figure 3.7). The quality analyses were performed after 24 hours and 1, 2, 3, 4 weeks.

Figure 3. 7 Samples prepared for storage

3.9 Physiochemical Analysis

3.9.1 Color Analysis

Color a* values, which indicate redness, were determined by colorimeter (Konica Minolta CR- 400, Japan). A thermal pasteurization control sample were included in all treatments. The a* value indicates redness-greenness with values varying scale between -60 (green) and +60 (red) Figure 3.8.

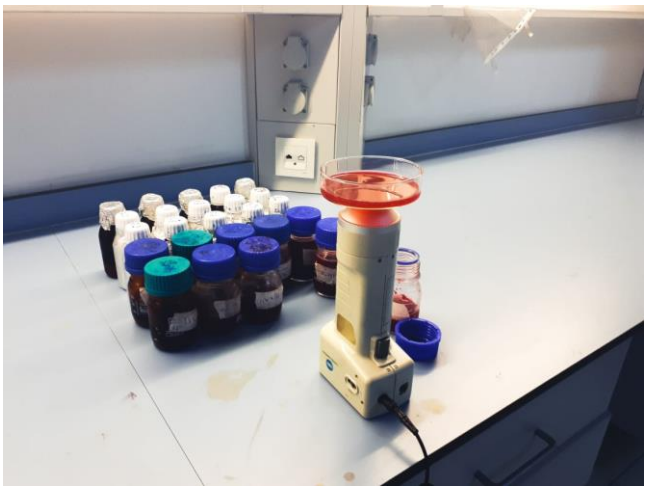


Figure 3. 8 Measure the color of the sample value a*

3.9.2 Brix Values Analysis and pH Value

°Brix values of the PJ were determined using the Abbe refractometer at 20 °C Figure 3. 9. pH amount of pomegranate juice were measured by first calibrating a pH meter (Thermo Scientific Orion Star A111, Indonesia) with standard buffered solutions. Then, the pH was determined by immersing the pH Figure 3. 10 electrode directly into the 22 ± 2 °C samples inside the beaker[191].

Figure 3. 9 Refractometer device

Figure 3. 10 Determination of the pH of pomegranate juice

3.9.3 Turbidity Analysis

To determine the turbidity value of pomegranate juice (PJ), 7 mL of the juice were combined with 1 mL of sterilized water and mixed gently. Then, the mixture's absorbance were measured by a spectrophotometer (Shimadzu UV-1800,Kyoto, Japan), at 660 nm[192].

3.9.4 Bioactive Properties.

3.9.4.1 Total Phenolic Content (TPC)

Total phenolic content (TPC) were specified by Singleton, Orthofer [193] as described earlier. The phenolic compounds are oxidized to phenolates via the reagent at alkaline pH in a saturated sodium carbon solvent, outcomes in a blue complex. For this aim, 0.5 mL of the sample were 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 mix. It then mixed for 1 min and left for a half-hour at room temperature in a dark condition. The absorbance values of the samples were calculated at 760 nm wavelength using a spectrophotometer device (Shimadzu UV-1800, Japan), against blank (distilled water) explain in Figure 3.11. Outcomes were expressed as milligrams of gallic acid equivalent per 1000 mL of sample weight (mg

GAE/1000 mL extract) and the formula used for calculating the total phenolic content was given in Equation 3.1.

$$TFC(mg/L) = ((Absorbance-0.0119)/0.0083) \times \text{Dilution Factor} \quad (3.1)$$

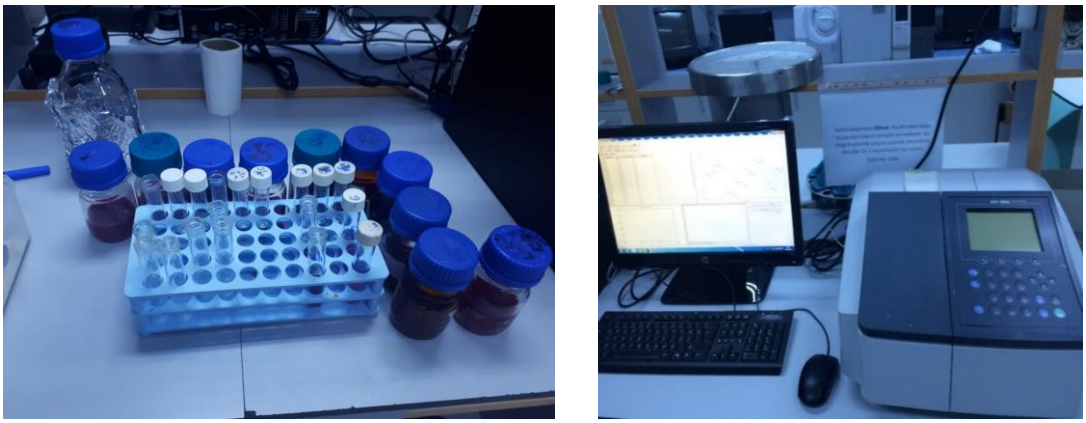


Figure 3. 11 Explain the measurement of the total phenolic content

3.9.4.2 Total Anthocyanin Content

The total anthocyanin content of the juice determined by considering the absorbance values measured at different pH values [194]. The absorption of the extracts were measured at 520 and 700 nm. The anthocyanin content of samples were calculated according to the following Equation 3.2.

0.025 M potassium chloride buffer, pH 1.0

0.4 M sodium acetate buffer, pH4.5

$$A = (A0 - A700)\ pH{:}1.0 - (A0 - A510)\ pH{:}4.5 \quad (3.2)$$

3.9.4.3 Antioxidant activity

Antioxidant activity were determined by a method described by Durak and Ucak [195]. This method determines the free radical scavenging activity of pomegranate juice. The 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical were used to test the antiradical activity of PJ. The DPPH solution (0.1 mM) were prepared in methanol. A 0.1 mL sample solution were added to each tube, and a 3.9 mL DPPH solution were incorporated and mixed with the vortex mixer for 1 min. The samples were incubating for 30 min in a dark environment, and the absorbance

were measured at 520 nm. The following Equation 3.3 calculated the antiradical activity (AA%) value.

$$\text{Scavenging activity (AA\%)} = (\text{A control-A sample}) \times 100 \quad (3.3)$$

3.9.4.4 Phenolic Profile by HPLC

The phenolic profile in PJ were determined by high-performance liquid chromatography (HPLC)[196]. 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 specimen 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 × 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 were 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 were returned to initial conditions. The flow rate were 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.

3.10 Microbiological Analysis

3.10.1 Determination of Total Yeast-Mold Count

The yeast and mold counts were conducted on DRBC agar using the spread plate technique. Aliquots of 10 mL were obtained from each pomegranate beverage after shaking fully at different time intervals during fermentation and storage. specimen were mixed with 90 mL of sterile peptone water, mixed very well, and

exposed to serial decimal dilutions from 1 to 10 of Ranger solution. Yeasts and molds were specified through plating on Sabouraud Chloramphenicol Agar after Keep them at a temperature 30 °C into Seventy-two hours, expressed log CFU/mL Figure 3. 12.

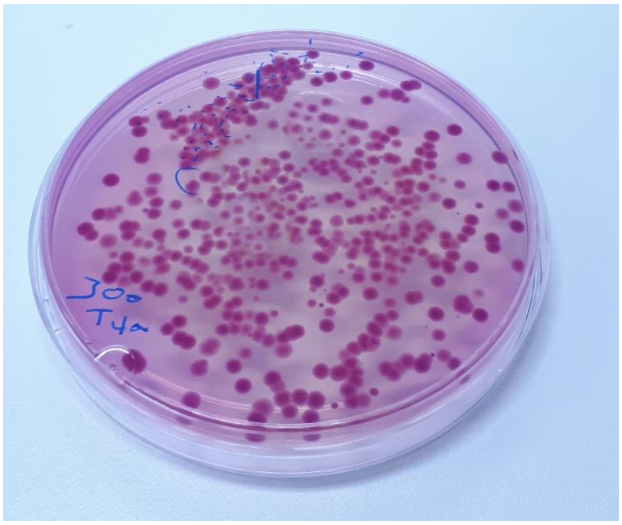


Figure 3. 12 Explain the amount of yeast and mold in microbiology analysis

3.10.2 Determination of Lactic Acid Bacteria (LAB)

Lactobacilli, yeasts, and fungi, and coliforms were specific in triplicate through plating suitable mitigation on the eclectic media for every species [197]. *Lactobacillus plantarum* especially counted within the MRS agar (Merck, Darmstadt, Germany) at temperature 37 °C for seventy-two hours Figure 3. 13.

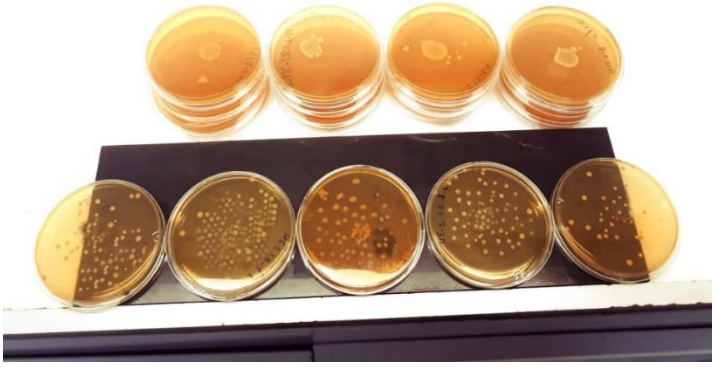


Figure 3. 13 The LAB colonies grown on MRS agar

3.10.3 Determination of Total Mesophilic Aerobic Bacteria

For determination of TMAB, appropriate dilutions were spread plated on Plate Count Agar (PCA), and the petri plates were incubated at 30 °C and 48 h. Then the colonies were counted Figure 3. 14.

Figure 3. 14 Microbial analysis

3.11 Sensory Analysis

A panel of 30 members performed a sensory review of the fermented pomegranate juice. They scored the taste, overall, and aroma acceptability in comparison with pomegranate drink after fermentation juices and during preservation at 5 °C [198]. The specimen were number through a various 3-digital numeral and were served in a randomized demand, while the panel were require to evaluate them determined a 0–10 preferred scale. The outcomes are given as medium outcome plus standard deviations Figure 3. 15.

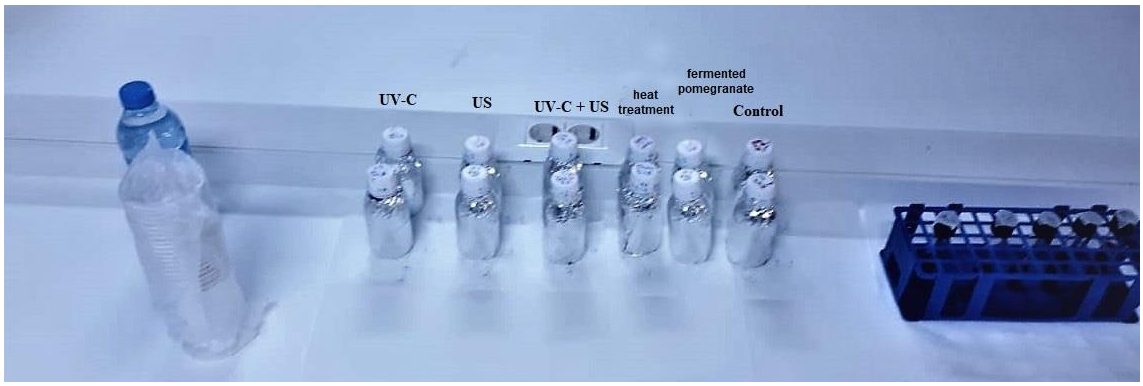


Figure 3. 15 Sensory analysis

3.12 Experimental Design and Statistical Analysis

Experimental design and 3D graphics were generated using the design-expert program version 7.0.0 (State-Ease Inc., USA). One-way analysis of variance (ANOVA) and Tukey tests using a Minitab V.17 statistical software (Minitab Inc., State College, PA, USA). Three independent variables were used to study the effects of (temperature, flow rate, and UV dose) in UV pasteurization. Similarly, the results of three independent variables were analyzed using a face-centered CCD response surface methodology (temperatures, ultrasound power on/off, and times) in the US pasteurization.

4

RESULTS AND DISCUSSION

4.1 Untreated Pomegranate Juice Sample

Table 4.1 shows the total phenolic content (TPC) of pomegranate juices. °Brix value, a* value, and yeast and mold content of untreated pomegranate juice.

Table 4. 1 Value of the TPC, Brix, turbidity, color, pH, and yeast and mold count of unprocessed pomegranate juice

TPC (mg GAE/L)	Turbidity	°Brix value	pH	Color a*	Yeast and mold count log CFU/mL
1078±5.72	4.2±0.19	14±0.2	3.2±0.1	2.3±0.1	5.3±0.1

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

4.2 Optimization of Parameters and Design Checks in UV Processing

According to the experimental design, the responses obtained for 12 samples with UV pasteurization are given in Table 4.2. The results showed that the TPC amounts, turbidity values, °Brix values, 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 °Brix, 2.3 to 2.5 a*, and <0.5 to 3.97 log CFU/mL, respectively.

Table 4. 2 Experimental parameters in the UV process and the observed response values for pomegranate juice

Run Order	X_1	X_2	X_3	TPC (mg GAE/L)	Turbidity	°Brix	Color a*	Yeast and Mold Count log CFU/mL
1	50	1.5	1 LAMP	920± 1.4 [g]	4.01± 0.1 [j]	14± 0.8 [c]	2.3± 0.3 [ab]	3.09± 0.9 [g]
2	60	1.5	1 LAMP	857± 0.4 [i]	3.59± 0.1 [k]	15± 0.8 [b]	2.3± 0.2 [ab]	<0.5 [k]
3	50	3.5	2 LAMPS	969± 0.5 [d]	3.99± 0.1 [g]	15± 0. 1 [b]	2.3± 0.1 [ab]	3.11± 0.1 [f]
4	40	1.5	1 LAMP	1055± 0.7 [ab]	4.58± 0.1 [b]	14± 0.8 [c]	2.3± 0.1 [ab]	3.75± 0.8 [c]
5	40	3.5	2 LAMPS	1053± 0.6 [b]	4.34± 0.1 [d]	14± 0.8 [c]	2.3± 0.2 [ab]	3.86± 0.5 [b]
6	50	3.5	1 LAMP	1020± 0.2 [c]	4.05± 0.1 [f]	15± 1.6 [b]	2.3± 0.1 [ab]	3.34± 0.5 [e]
7	50	1.5	2 LAMPS	890± 0.5 [h]	4.19± 0.1 [e]	15± 0.8 [b]	2.3± 0.2 [ab]	2.97± 0.6 [h]
8	60	1.5	2 LAMPS	840± 0.6 [j]	3.61± 0.1 [k]	16± 0.1 [a]	2.5± 0.1 [ab]	<0.5 [k]
9	40	3.5	1 LAMP	1060± 4.01 [a]	4.41± 0.1 [c]	14± 0.1 [c]	2.3± 0.2 [ab]	3.92± 0.56 [a]
10	40	1.5	2 LAMPS	938± 1.4 [f]	4.61± 0.1 [a]	14± 0.1 [c]	2.3± 0.2 [ab]	3.59± 0.50 [d]
11	60	3.5	1 LAMP	958± 5.9 [e]	3.82± 0.1 [h]	15± 0.0 [b]	2.3± 0.1 [ab]	2 ± 0.00 [i]
12	60	3.5	2 LAMPS	925± 0.66 [g]	3.76± 0.1 [i]	15± 0.0 [b]	2.5± 0.1 [a]	1.69± 0.82 [j]

X_1 (Temperature, °C), X_2 (Flow rate, L/min), X_3 (UV Lamp 1 and 2 doses are 5.1 and 10.1 mW/cm2, respectively). Mean with superscripts of different small letters in the same columns are significantly different as Tukey's 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 factors with the responses are given in Table 4. 3. The results showed that temperature and flow rate parameters were statistically significant ($p<0.05$) for the amount of yeast and mold, and the UV dose were not statistically significant. Considering the results, the UV dose increase 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 Figure 4.1.

Since the microbial growth were already undetectable at this temperature, the UV dose's intensity were statistically insignificant. UV treatment resulted in an

average of 1-2 log reduction of the yeast and mold count. The value of R^2 were 0.999, the responses are very near to 1, showing a strong linkage between the independent parameters and the answers.

Table 4. 3 ANOVA analysis results of factorial Design for yeast and mold content in the 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_1X_2	1.79	0.89	85.07	0.0116
X_1X_3	0.00	0.00	0.11	0.9046
X_2X_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)

Regarding the total phenol content of UV-treated pomegranate juice, where the temperature were statistically significant ($p<0.05$) for the TPC content and flow rate, and UV dose parameters and the interaction of the factors with the responses were not statistically significant ($p>0.05$) at ANOVA analysis. Even though the interaction of the factors temperature values, flow rate, and UV dose in the UV pasteurization process for TPC were not statistically significant, but it was observed that the value of the total phenol content of juice decreases upon higher treatment temperatures Figure 4.2.

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 were 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) and the lowest flow rate (1.5 L/min) inhibited almost all yeast and mold growth. 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. Also, 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, it was determined that microbial growth could be minimized at 50 °C with a 1.5 L/min flow rate and when 2 lamp UV doses were applied. Desirability, which is a criterion for accuracy in optimization processes, were found to be 1.000.

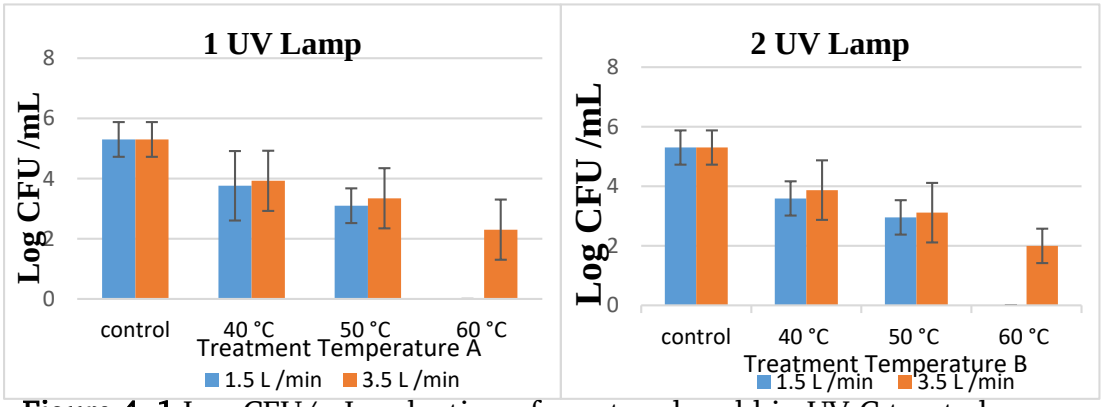


Figure 4. 1 Log CFU/mL reduction of yeast and mold in UV-C treated pomegranate juice(A: represents the use of 1 lamp UV, B: represents 2 lamp UV)

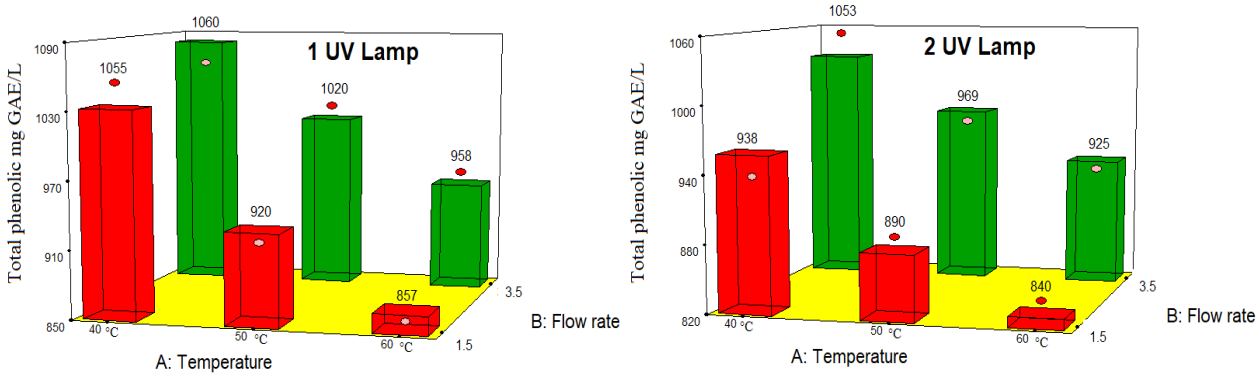


Figure 4. 2 Change in total phenolic in UV-C treated pomegranate juice when using 1 lamp UV and 2 lamp UV

Pomegranate fruit (*Punica granatum L.*) is a source of bioactive compounds such as ellagic acid, punicalagin, ellagitannins, etc. [72], [196]. Thermal pasteurization of pomegranate juice generally leads to a decrease in the bioactive compounds of fresh juice. Studies show that ultraviolet light's use to pasteurize fresh juices affects the sample's phenolic content and deteriorates depending on time and dose [199]. Our study reported a decrease in the TPC amount of PJ samples compared to unprocessed juice. These results are close to previous research that the UV-C applied to fresh apple juice [200]. Besides, Pala and Toklucu [2] 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 were stated that long-time UV treatment reduces the number of phenolic substances [201].

On the other hand, the lower yeast and mold count were observed using high UV and long-time UV treatment doses. The reduction of yeast and mold count varied 1 to 2 log when used different doses and time for exposure. These results had statistically significant similarity with a study conducted by Pala and Toklucu [2], who reported 1 log reduction of yeast and mold to count when used 34,4 j/Ml and 62,4 j/ml UV. Similarly, The UV-C were applied to grape and cranberry juice inoculated with *Saccharomyces cerevisiae* ATCC 10274 and reported 0.53 log and 2.5 log reduction [202]. In another study, the UV-C were applied to the strawberry juice by Keyser, Műller [161], and the reduction for yeast and mold were 2.45 log CFU/mL. The °Brix value increased due to the increase in temperature, which is statistically significant ($p<0.05$).

In contrast, 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 the amount of water decreases by evaporation and the amount of dry matter soluble in water increases proportionally.

Our results are similar to the research paper published by Rivas, Rodrigo [203]. He mentioned that °Brix value of heat pasteurization applied blended orange and carrot juice increased significantly, compared with untreated juice. Concerning

the value of turbidity in pomegranate juice treated by ultraviolet light, 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 can promote the solubility of dispersed compounds. Few studies in the literature 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 [169], [201], [204].

4.3 Optimization of the Parameters and Verification of Design in US Processing

According to the CCD, the responses obtained from 18 experimental points with US pasteurization are shown in Table 4.4. For 18 experimental points, the values of TPC, turbidity, °Brix, color a*, and yeast and mold count ranged from 1080 to 1481 mg GAE/L, 3.88 to 4.71 NTU, 14 to15 °Brix, 3.03 to 3.88 Color a*, and 0 to 4.8 log CFU/mL, respectively. 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 4. 5. The results showed that the interaction factor between temperature, time, and ultrasound on/off parameters were statistically significant for the yeast and mold content. Increasing the temperature and processing time reduced yeast and mold count. US power-off means no ultrasound treatment were used 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 ($p<0.05$) Figure 4.3.

As seen in Table 4.5, The interaction factor between 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 Figure 4.4. The R^2 value were 0.9745 for the TPC responses, which were very close to 1 and showed a strong correlation between the independent parameters and responses. The turbidity value interaction factor between the parameters of temperature,

time, and the US on/off position were statistically significant ($p<0.05$). °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 an undetectable level. The increase in temperature and time factors, and the contribution of ultrasonic treatment significantly increased the amount of TPC. Also, 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, it determined that yeast and mold content could be minimized when the temperature is 50 °C, and the treatment time is 15 min, and the US power-on position were applied. Desirability were also found to be 0.802.

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

Run Order	X_1	X_2	X_3	TPC (mg GAE/L)	Turbidity	°Brix	Color a*	Yeast and Mold Count log CFU/mL
1	50	10	ON	1252 ± 1.5 ^{d}	4.23 ± 0.1 $_{bcde}$	15 ± 0.8 ^{a}	3.46 ± 0.1 ^{f}	3.4 ± 0.8 ^{i}
2	40	10	OFF	1103 ± 0.2 ^{gh}	4.11 ± 0.1 ^{h}	15 ± 0.8 ^{a}	3.46 ± 0.1 ^{f}	4.4 ± 0.5 ^{a}
3	50	15	ON	1273 ± 0.1 ^{d}	4.24 ± 0.1 ^{bcd}	15 ± 0.8 ^{a}	3.46 ± 0.1 ^{f}	3.0 ± 0.9 ^{k}
4	40	15	ON	1138 ± 0.1 ^{f}	4.20 ± 0.1 ^{def}	15 ± 0.8 ^{a}	3.54 ± 0.1 ^{c}	4.0 ± 0.8 ^{e}
5	60	5	ON	1310 ± 0.1 ^{c}	4.28 ± 0.1 ^{b}	15 ± 0.8 ^{a}	3.53 ± 0.1 ^{d}	3.1 ± 0.8 ^{j}
6	60	10	ON	1358 ± 0.1 ^{b}	4.28 ± 0.1 ^{b}	15 ± 0.8 ^{a}	3.16 ± 0.1 ^{h}	2.8 ± 0.56 $_{l}$
7	60	5	OFF	1173 ± 0.1 ^{e}	4.25 ± 0.1 ^{bcd}	14 ± 0.8 ^{b}	3.03 ± 0.1 ^{j}	3.9 ± 0.8 ^{f}
8	50	5	OFF	1116 ± 0.1 ^{fg}	4.14 ± 0.1 ^{fgh}	14 ± 0.8 ^{b}	3.53 ± 0.1 ^{d}	4.1 ± 0.8 ^{c}
9	40	15	OFF	1119 ± 0.1 ^{fg}	4.13 ± 0.1 ^{gh}	15 ± 0.8 ^{a}	3.46 ± 0.1 ^{f}	4.2 ± 1.2 ^{c}
10	40	5	OFF	1080 ± 0.1 ^{h}	3.88 ± 0.1 ^{j}	14 ± 0.8 ^{b}	3.47 ± 0.4 ^{e}	4.8 ± 0.4a
11	60	15	ON	1481 ± 0.1 ^{a}	4.71 ± 0.1 ^{a}	15 ± 0.8 ^{a}	3.16 ± 0.1 ^{h}	<0.5 ^{m}
12	60	10	OFF	1173 ± 0.2 ^{e}	4.26 ± 0.1 ^{bc}	15 ± 0.8 ^{a}	3.16 ± 0.1 ^{h}	3.9 ± 0.7 ^{g}
13	60	15	OFF	1192 ± 0.1 ^{e}	4.28 ± 0.1 ^{b}	15 ± 0.8 ^{a}	3.17 ± 0.1 ^{g}	3.8 ± 0.5 ^{h}
14	40	10	ON	1121 ± 0.2 ^{fg}	4.18 ± 0.1 ^{efg}	15 ± 0.8 ^{a}	3.54 ± 0.1 ^{c}	4.3 ± 1.41 $_{b}$
15	40	5	ON	1082 ± 0.2 ^{h}	4.03 ± 0.1 ^{i}	15 ± 0.8 ^{a}	3.05 ± 0.1 ^{i}	4.7 ± 0.5 ^{a}
16	50	15	OFF	1123 ± 0.16 $_{fg}$	4.23 ± 0.1 $_{bcde}$	15 ± 0.8 ^{a}	3.88 ± 0.1 ^{a}	3.92 ± 0.8 $_{f}$
17	50	5	ON	1140 ± 1.8 ^{f}	4.14 ± 0.1 ^{fgh}	15 ± 0.8 ^{a}	3.16 ± 0.1 ^{h}	3.8 ± 1.6 ^{h}
18	50	10	OFF	1124 ± 1.8 ^{fg}	4.21 ± 0.1 ^{cde}	15 ± 0.8 ^{a}	3.63 ± 0.1 ^{b}	4.0 ± 1.2 ^{d}

X_1 (Temperature, °C), X_2 (Time, min), X_3 (OFF: no US treatment; ON: US treatment) Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3)

Table 4. 5 ANOVA analysis results of central composite design (CCD) for yeast and mold count in the 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_1X_2	0.91	0.91	3.64	0.0889
X_1X_3	2.61	2.61	10.43	0.103
X_2X_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 the microbial analysis process (log CFU/mL)

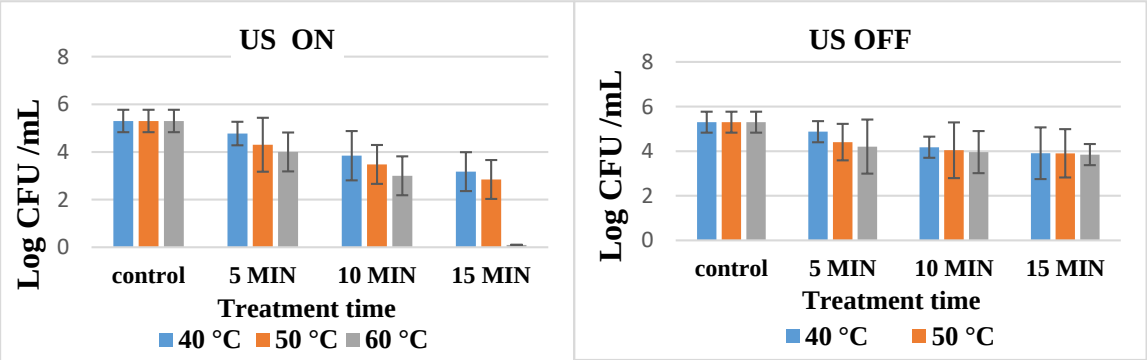


Figure 4. 3 Log CFU/mL reduction of yeast & mold in ultrasound treated pomegranate juice when the ultrasound is on or off

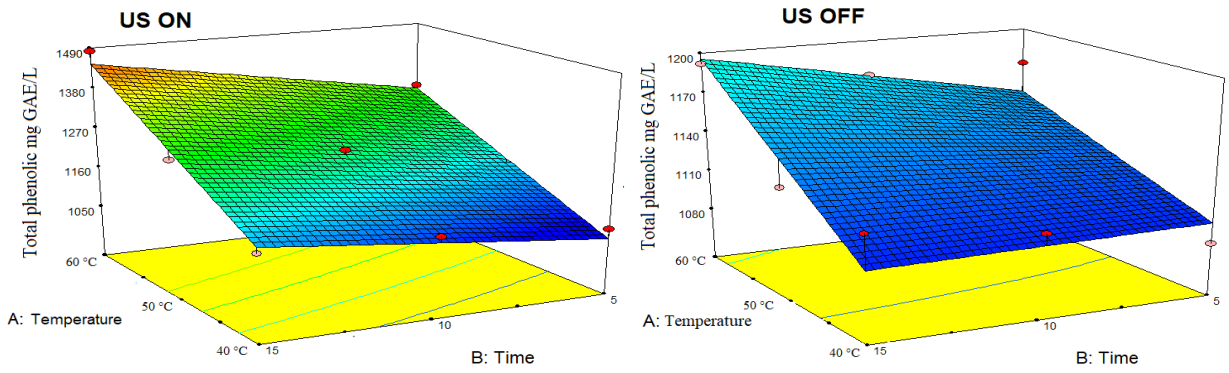


Figure 4. 4 Change in total phenolic in ultrasound treated pomegranate juice when the ultrasound is on or off

Some studies have been conducted on using sonication in food products to inhibit *S. cerevisiae* and bacteria by using ultrasound model systems [15], [205]. In this 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 can be explained with improvements in the extraction efficiency of the ultrasound process.

This process might help release the bound form of phenolic acids with the help of the cavitation effect. These results obtained from our study are consistent with other researches [176], [177]. The US treatment resulted in an average 1–2 log reduction of the yeast and mold count. The yeast and mold count obtained at low temperature, and the US power-off position were close to each other, and the reason is that increasing temperature and the US treatment affect the growth of microorganisms.

Our results are comparable or similar to a previous study that observed the inhibition effect of ultrasound treatment on *Saccharomyces cerevisiae* in pomegranate juice [13]. There are 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 [206] in red grape juice and in blackberry juice by Tiwari, O'Donnell [14].

4.4 Optimization Parameters 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. The optimum condition was 50 °C for temperature, 15 min for treatment time, and US power-on position for the US treatment. After determining the optimum condition for UV and US treatment, these two processes were combined, and the PJ were examined in terms of the results of TPC, turbidity, °Brix, and a*, and yeast and mold count. In consideration of stand-alone optimum conditions, the experimental design for the UV+US process were generated to obtain the optimum combined condition. On the other hand, when the experimental design for the UV+US combined process

were carried out with 2 lamps and 1.5 L/min UV application, the yeast and mold count responses were 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 were decreased to 1 lamp considering the responses obtained from the experimental design. In this way, the condition for flow rate and the number of lamps were modified. Under these circumstances, the optimum condition for UV+US were also carried out at different experimental points and evaluated by an expert design program. The study results under these conditions can be observed from the values given in (Table 4.6).

Combined US+UV application at 40 °C were 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 than the conventional pasteurization method, and the TPC of PJ were also mostly preserved. Depending on increasing temperature and treatment time, the 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 conclusion, the optimum condition for UV+US combined process were the application of the temperature at 50 °C, UV 1 lamp on, and flow rate of 3.5 L/min for 10 min in the US on mode.

Table 4. 6 Experimental parameters in US+UV pasteurization of 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 (Ultrasound ON), X4 (UV 1 Lamp dose is 5.1 mW/cm2), X5 (UV Flow rate, L/min). Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3)

4.5 Comparison of UV, US, and UV+US Pasteurization Applications with Conventional Pasteurization

The quality parameters of untreated samples treated with conventional pasteurization, UV, US, and UV+US pasteurization processes are given in (Table 4.7). 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 at15 seconds. The US, UV, and combined US+UV treatments were applied at 50 °C. For each technique, it was used previously obtained optimum condition 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 the 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 that applied the conventional pasteurization process. The best pasteurization method for saving anthocyanin content in the samples were 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, US and UV+US treatment increased the number of phenolic compounds. Turbidity for unprocessed and pasteurized samples were 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 were observed compared to PJ applied conventional pasteurization.

Table 4. 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 ± 0.01 ^{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}
p–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 ± 0.1 ^{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}

Mean with superscripts of different small letters in the same rows are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3)

4.6 Fermentation of the Pomegranate Sample Resulting from Combining Two Methods of Pasteurization by Ultraviolet Radiation and Ultrasound by Bacteria *Lactobacillus plantarum*

When determining the optimal conditions for the pasteurization processes by using the two techniques, ultraviolet radiation and ultrasound together, were fermented the pomegranate drink sample via lactic acid bacteria *Lactobacillus plantarum* and observed the physicochemical changes that occur in the selected sample for 28 days at a temperature of 5 °C.

4.6. Cell growth of Bacteria *Lactobacillus plantarum* of Ferment Pomegranate Syrup

The viability of *Lactobacillus plantarum* and potential spoilage by mold and yeasts or aerobic mesophilic bacteria were shown after the fermentation process for the juice during the four weeks of storing the juice at a temperature 5 °C (Table 4.8, 4.9). In accordance with the outcomes, the probiotic *Lactobacillus plantarum* strain's cell viability were preserved at greatly levels during 3 weeks of cold storage above 9 CFU/mL, whilst declined throughout the latest week of storage statistically significant ($p<0.05$). Particularly, viable probiotic cell counts were reduced to 7.11 log CFU/mL in the fourth week of storage Figure 4.5. Nevertheless, in this situation, where the products are called probiotics, there must be the value of lactic acid bacteria greater than 6 to 7 CFU/mL, which is necessary for probiotic produce [69]. At this time, it must be noted emphasized The initial pH amount of the fresh pomegranate drink used in this research was approximately 3.2.

A possible explanation of *Lactobacillus plantarum* viability has raised levels when preservation is lactic acid fermentation possibility to be increasing the bioaccessibility of phenolic compounding. There was papers from the literature allegation that phenolic compound maybe act as prebiotics [207]. Moreover, potential prebiotic act led to the amelioration of the increasing of *Lactobacillus plantarum*. Also maybe observed that some strains of *Lactobacillus plantarum* can grow in some fruits due to their ability to withstand acidic environments [208]. Furthermore, no spoilage no damage to the fermented pomegranate juice were

observed by yeast and mold, and Mesophilic Aerobic Bacteria even after the fourth week of Preservation at a temperature 5 °C. From days 0 until the end of storage (28 days), no growth of Mesophilic Aerobic Bacteria (<10 CFU/mL) were observed in all processed samples (for UV, US, UV+US, control) the sample is thermally treatment. No growth of mold plus yeasts (<10 CFU/mL) is observed in the UV+US and thermally processed pomegranate beverages. The values of yeast and mold respectively the first days to 28 days at end of storage for UV, US, and control sample beverages were 2.90±1.2, 4.01±1.6 and 3.0±1.6, 4.40±0.8 and 5.30±0.8, 6.82±0.8 log CFU/mL, as noted in Table 4. 9.

There were significant yeasts and mold changes during the storage period in the pomegranate juice sample pasteurized by ultraviolet radiation. The increase at the end of storage reached 1.1 logs. As for the number of yeasts and mold in the sample of pomegranate juice pasteurized by ultrasound, there were significant changes as the percentage of increase during the storage period reached 1.3 log (Figure 4.6). It appears that pomegranate juice fermented by lactic acid It can provide protection from microbiological damage, as mentioned earlier studies [209], [210].

Table 4. 8 Cell viability of bacteria *Lactobacillus plantarum* in the fermented pomegranate juice after twenty four hours in 30 ° C and preservation at 5°C

Temperature (^{O}C)	Viability *Lactobacillus plantarum* (log CFU/mL)		
	Time	*Lactobacillus plantarum*	Mesophilic Aerobic Bacteria
30	Twenty four hour	9.64 ± 1.5 a	<0.5±00. ^{aD}
5	First week	9.5 ± 1.1 a	<0.5±0.0 ^{aD}
5	Second week	9.11 ± 0. 6 a	<0.5±0.0 ^{aD}
5	Third week	9 ± 1 a	<0.5±0.0^{aD}
5	Fourth week	7.11 ± 1 b	<0.5±0.0^{aD}

Superscript similar letters indicate that there are no statistically significant differences = 0.05 (ANOVA, Tukey's test Post Hoc Multiple Comparisons)

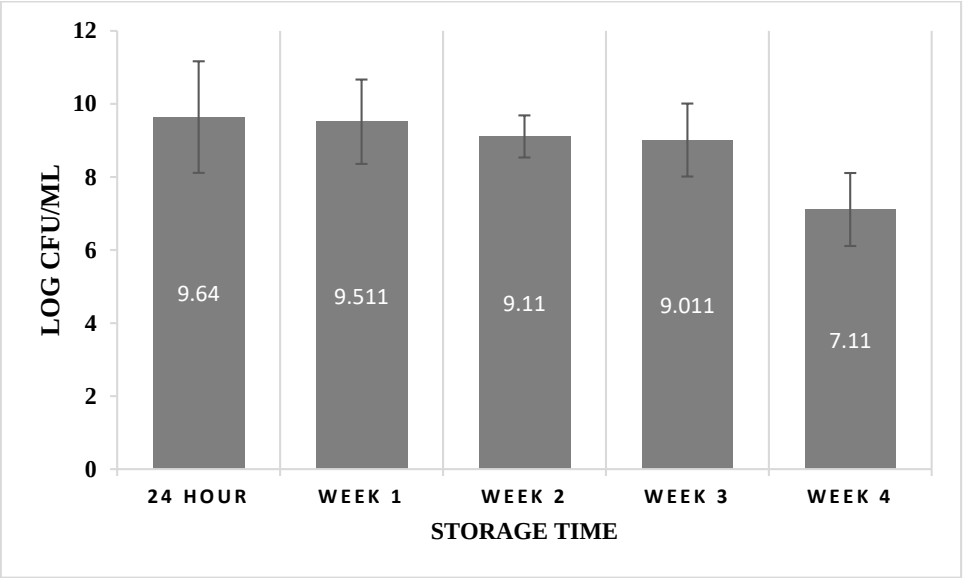


Figure 4. 5 Viability bacteria *Lactobacillus plantarum* in the fermented pomegranate juice After twenty-four hours of fermentation at a temperature in 30 °C and preservation at 5 °C

Table 4. 9 Viability of the mold plus yeasts counts in UV, US, UV+US, untreated sample, thermally pasteurized, and fermented samples along the storage time

Treatment Sample	Viability Yeast and Mold (log CFU/mL)				
	24 hours	1 week	2 weeks	3 weeks	4 weeks
UV	2.90 ± 1.2^{eC}	3.08 ± 0.8^{dC}	3.60 ± 0.8^{cC}	3.81 ± 1.0^{bC}	4.01 ± 1.6^{aC}
US	3.00 ± 1.6^{eB}	3.30 ± 1.6^{dB}	3.81 ± 1.4^{cB}	4.02 ± 0.8^{bB}	4.40 ± 0.8^{aB}
UV+US	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$
Control	5.30 ± 0.8^{eA}	5.74 ± 0.7^{dA}	6.00 ± 0.8^{cA}	6.64 ± 1.2^{bA}	6.82 ± 0.8^{aA}
Thermal	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$
Fermented pomegranate	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$	$<0.5\pm0.0^{aD}$

Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3).

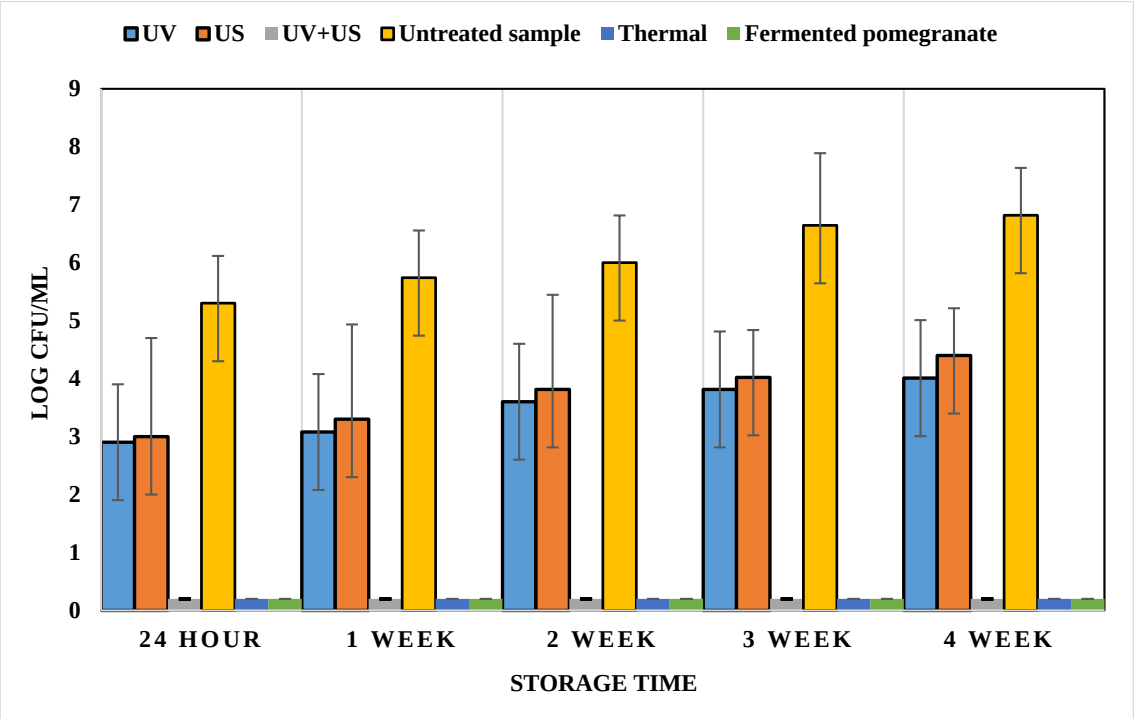


Figure 4. 6 Microbial value for the fermented sample and non-fermented samples UV, US, ultraviolet, ultrasound, and through preservation at 5 ± 1°C for four weeks

4.6.2 Antioxidant Activity

The amount of antioxidant activity of fermented pomegranate drink, mainly the initial antioxidant activity of the ideal selected sample obtained from a combination of non-thermal pasteurization methods, were about 27.08±0.1 milligrams TE/100 mL. Through in the first wenty-four hours, the antioxidant activity of the fermented pomegranate drink was a significant change ($P < 0.05$) increased to an average of 43.4±0.4 mg TE/100 mL, in comparison with the specific value of the optimum point for UV+ US treatment non-fermented juice which represents the sample control that were reduced to 27.04±0.4 mg TE/100 mL after 24 hours of storage. The antioxidant activity of fermented pomegranate juice increased in all the weeks of preservation time, which is statistically significant ($p < 0.05$), where the value of antioxidants reached in the 2nd week to (60.5±1.12mg TE/100 mL), and the antioxidant value were reached in the 4th week at the end of storage to (54.15±1. 31mg TE/100 mL) Table 4.10. In contrast, the antioxidant activity of non-fermented pomegranate juice and

optimum point for UV+ US treatment non-fermented juice were respectively continuously decreased to 28.85±2.29 and 23.54±2.28 TE/100 mL in the fourth week of the storage process. A potential clarification of this decision is that lactic acid fermentation improved antioxidant activity of pomegranate juice, which is reported in the literature [23]. In particular, other scientists have shown that some bacteria can generate β-galactosidase, catalyzing the releasing of bonded sugar phenolic compounds. [211]. This process may increase the activity of antioxidants within a week of fermentation [212], [213].

The antioxidant capacity (AC) results are shown in Figure 4.7, a significant difference decrease ($p \leq .05$) were observed in the antioxidant activity content within the UV, US, UV+US, control, and thermal pasteurized processed beverages. The antioxidant capacity values after 24 h to 28-days end of storage for UV, US, UV+US, control, and thermal were 27.52±6.06 and 22.76±2.1, 31.61±4.7 and 26.77±2.1, 27.04±4.05 and 23.54±2.28, 31.06±3.73 and 28.85±2.29, 26.67±5.849 and 20.76±1.03, mg Trolox/100 mL of beverage respectively. The beverage with the highest loss of AC were the thermal pasteurized pomegranate beverage: after 24 h, it had 23.54±2.09 mg Trolox/100 mL, and on days 28, it had 20.76±1.03 mg Trolox/100 mL; this could be due to the heat and the longer time used for pasteurization [62]. Plaza et al. (2006) found a more significant reduction of antioxidant capacity in pasteurized (70 °C at 30 s) orange juice than in samples processed at 400 MPa for 1 min and stored 40 days at 4 °C. The results demonstrated better retention of antioxidants than the heat treatment is UV+US pasteurization for the non-fermented sample. However, the best sample is the fermented sample it has antioxidants of a great value compared to the rest of the samples.

Besides, the increase or decrease in antioxidant activity could be due to a combined effect of different compounds, which act synergistically or antagonistically. A series of factors that influence the antioxidant activity, such as the oxidation system, the degree of glycosylation, the partition coefficient, and the concentration of other antioxidant compounds that the fruit could have, could be correlated with the antioxidant activity [214], [215].

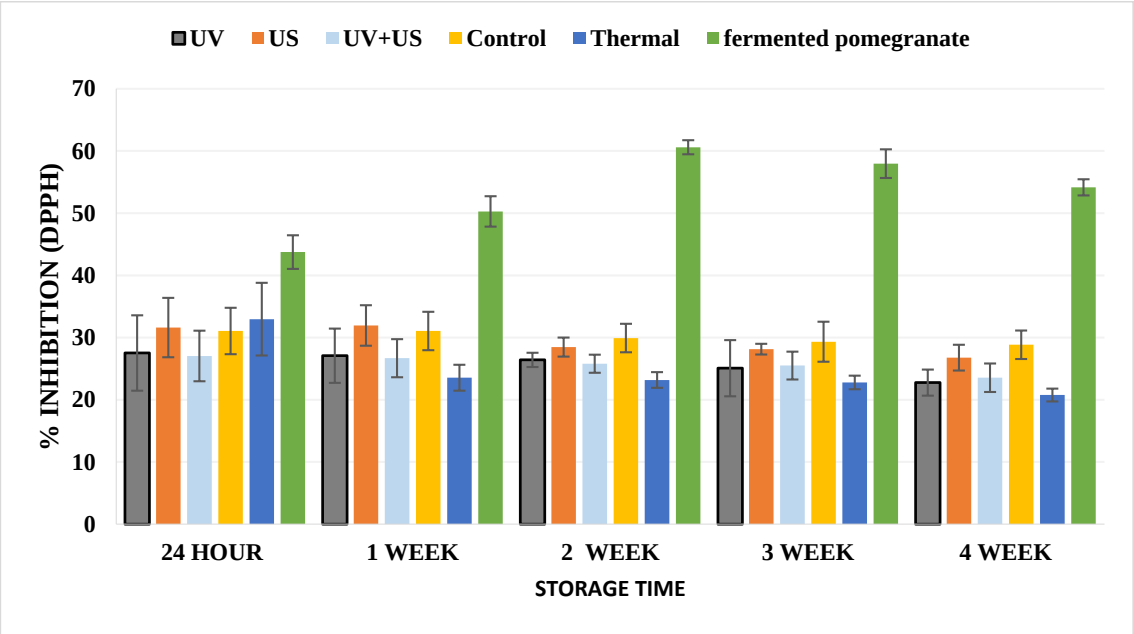


Figure 4. 7 Antioxidant activity of fermented sample with *Lactobacillus plantarum* and non-fermented sample and UV, US, UV+US, and control pomegranate juice through preservation at a temperature 5 °C for fourth weeks. TE, trolox equivalent

Table 4. 10 Antioxidant activity of fermented sample and non-fermented sample and UV, US, UV+US and control pomegranate juice through preservation at temperature 5 °C for fourth weeks. TE, trolox equivalent

Treatment Sample	24 hours	1 week	2 weeks	3 weeks	4 weeks
UV	27.5 ± 6.1^{aA}	27 ± 4.3^{abB}	26.4 ± 1.1^{abcAB}	25 ± 4.5^{bcC}	22.7 ± 2.1^{cC}
US	$31.6\pm4.\ 7^{aA}$	31.9 ± 3.2^{aA}	28.4 ± 1.5^{aA}	28.1 ± 0.8^{aB}	26.7 ± 2.1^{aB}
UV+US	27 ± 4.1^{aA}	26.6 ± 3.1^{aB}	25.7 ± 1.4^{aAB}	25.5 ± 2.2^{aC}	23.5 ± 2.3^{bC}
Control	31 ± 3.7^{aA}	31 ± 3.1^{aA}	29 ± 2.3^{aA}	29.3 ± 3.2^{aA}	28.8 ± 2.3^{aA}
Thermal	26.6 ± 5.8^{aA}	23.5 ± 2.1^{abC}	23.1 ± 1.2^{abB}	22.7 ± 1.1^{abD}	20.7 ± 1.1^{bD}
Fermented pomegranate	43.7 ± 2.7^{aA}	50.2 ± 2.5^{abC}	60.5 ± 1.1^{aAB}	57.9 ± 2.3^{aB}	$54.1\pm1.\ 3^{bC}$

Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3).

4.6.3 Total phenolic content

The values of TPCs at after 24 h to 28-days end of storage for fermented sample like outcomes were discovered as in the case of antioxidant activity and UV, US, UV+US, control, and thermally processed beverages were 1460.96 and 1507.4, 890.24, and 700.78, 1273.07 and 986.44, 1262.17 and 1061.63, 1078.07 and 902.1, 889.94 and 773.25 (mg GAE/L) shown in Figure 4. 8. The decrease of total phenolic content among all treatment beverages until the end of storage were statistically significant ($p < 0.05$).

The initial total phenolic content of the juice selected from the combination of the two pasteurization methods were about 1263 ± 4.1 mg GAE/100 mL. Then this juice fermented by *Lactobacillus plantarum*. The total phenol content of the fermented pomegranate juice increased, which is statistically significant ($p < 0.05$); within 24 hours the percentage reached 1460.96 mg GAE/100 mL after 24 hours of fermentation compare to the respective amount of the UV+US non-fermented juice which represents a sample control that were decreased to 1262.17mg GAE/100 mL. The largest loss in the total phenol content in heat pasteurized pomegranate juice, as the total phenol content at the last week of storage, were 773.25±1.05 GAE/100 mL.

The fermented pomegranate juice had a high content of the total phenol content during the four storage weeks compared with the samples treated by ultraviolet radiation, ultrasound, and the sample pasteurized by heat and the control sample. The highest value obtained in the second week of storage for the sample fermented pomegranate juice with bacteria *Lactobacillus plantarum*. The total phenol content evaluated in the second week 1635.7 mg GAE/100 mL. While TPC of the UV+US non-fermented pomegranate juice (control) decreased to 1061.63mg GAE/100 mL at the 4th week of storage, this decrease were statistically significant ($p < 0.05$). In the literature, lactic acid fermentation has been reported to improve fruit juices' total phenolic content, including pomegranate juices[216], [217]. Other researchers who have demonstrated the same outcome reported that improvements in TPC of pomegranate juice could be related to the increase in the free form of phenolic compounds through the fermentation and the product of

new phenolic derivatives such as catechin and α-punicalagin [19], [27], [207], [218]. The UV+US beverage showed significant differences ($p \leq .05$) in the content of TPCs compared to control, UV, US, and thermal. The sample fermented by bacteria had a high content of phenolic compounds during the storage period compared to other samples. The reduction rates of TPCs content are reported in Table 4. 11. Results were close to a study published for pomegranate juice when pasteurized by thermal pasteurization and stored for 48 days, seeing the physiochemical differences that occur during the storage period and comparing it with untreated juice [219].

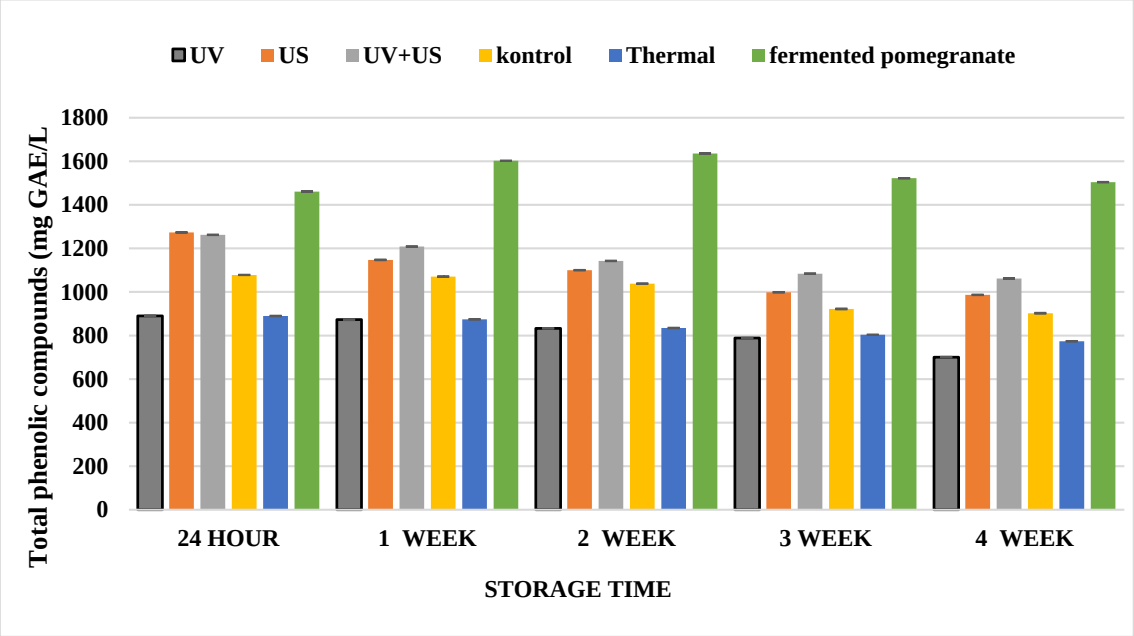


Figure 4. 8 Total phenolic content of fermented sample with *Lactobacillus plantarum* and non-fermented sample and UV, US, UV+US and control pomegranate juice through preservation at temperature 5 °C for four weeks. TE,trolox equivalent

Table 4. 11 Total phenolic content of fermented and non-fermented pomegranate juice UV+US, ultraviolet, ultrasound, and through preservation at temperature 5 ± 1°C for four weeks

Treatment Sample	24 hours	First week	Second week	Third week	Fourth week
UV	890.2±2.1 ^{aD}	873.07±1.8^{bD}	832.9±1.1^{cD}	788.3±1.1 ^{dD}	700.7±1.1 ^{eD}
US	1273±1.0 ^{aB}	1147.2±1.2^{bB}	1099.7±1.0 ^{cB}	998.3±0.9 ^{dB}	986.4±1.1 ^{eB}
UV+US	1262.1±0.9 ^{aB}	1208.3± 0.9 ^{bB}	1142.4± 1.2 ^{cB}	1084±1.8 ^{dB}	1061.6±1.2 ^{eB}
Control	1078±0.8 ^{aC}	1070.4±0.7 ^{bC}	1038.1±1.0 ^{cC}	921.8±1.8 ^{dC}	902±1.6 ^{eC}
Thermal	889.9±1.0 ^{aD}	874.2±1.0 ^{bD}	834.5±1.0 ^{cD}	803.4± 0.9 ^{dD}	773.2±1.1 ^{eD}
Fermented pomegranate	1460.9±0.7 ^{aA}	1602.5±0.8 ^{bA}	1635.7±1.7 ^{cA}	1522± 0.8 ^{dA}	1504.2± 1.1 ^{eA}

Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3)

4.6.4 Anthocyanins Content

The anthocyanins content of the fermented pomegranate juice samples and the unfermented samples were no significant changes during all four weeks of storage like has been reported in the literature that no significant changes in anthocyanins content during storage [219]. A significant decrease of anthocyanins content was observed for pomegranate juice pasteurized by thermal from 17.97 ± 0.37 to 16.97 ± 0.6 mg 100 mL until the end of storage time in the fourth last weeks. Results showed that the anthocyanins content values of untreated pomegranate juice (control) were higher than the UV, US, UV+US, thermal, and fermented juice samples.

However, storage time were found to decrease the anthocyanins content for all samples, but no significant changes ($P > 0.05$). It is known that anthocyanins are highly unstable to physical factors such as light, oxygen, pH, and temperature. The anthocyanins content from 24h to the 28-days end of storage for UV, US, UV US, control, thermal processed, and fermented sample beverages were 25.85 and 20.48, 20.62 and, 20.03, 20.7 and 20.37, 25.13 and 24.44, 17.97 and 16.97, 20.23

and 20.37 mg 100 mL respectively Table 4.12. However, it must be taken into account that pressure, temperature, and time of processing and physicochemical properties such as TSSs, pH, and acidity could affect the enzymes responsible for the stability of anthocyanins in food products [220].

Table 4. 12 Anthocyanins value fermented and non-fermented pomegranate juice UV+US, ultraviolet, ultrasound, and through preservation at temperature (5 ± 1 °C) for four weeks

Treatment sample	24 hours	1 week	2 weeks	3 weeks	4 weeks
UV	20.8 ± 0.4^{aB}	20.8 ± 0.4^{aB}	20.7 ± 0.3^{aB}	20.6 ± 0.1^{aB}	20.4 ± 0.3^{aB}
US	20.6 ± 0.1^{aB}	20.5 ± 0.1^{aB}	20.4 ± 0.3^{aB}	20.2 ± 0.4^{aB}	20 ± 0.6^{aB}
UV+US	20.7 ± 0.9^{aB}	20.6 ± 1.0^{aB}	20.3 ± 1.0^{aB}	20.3 ± 0.4^{aB}	20.3 ± 0.4^{aB}
Control	25.1 ± 0.1^{aA}	25 ± 0.2^{aA}	24.8 ± 0.3^{aA}	24.6 ± 0.5^{aA}	24.4 ± 0.9^{aA}
Thermal	17.9 ± 0.4^{aC}	17.5 ± 0.1^{aC}	17.3 ± 0.2^{aC}	17.1 ± 0.4^{aC}	16.9 ± 0.6^{aC}
Fermented pomegranate	20.2 ± 0.9^{aB}	20 ± 1.0^{aB}	20.3 ± 0.1^{aB}	20.3 ± 0.1^{aB}	20.3 ± 0.1^{aB}

Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3)

4.6.5 Brix Analysis

Pomegranate juice characteristics regarding the Brix value during UV, US, UV+US, thermal treatment, and control are listed in Table 4.13. No significant changes ($p < 0.05$) in °Brix value was observed between the fermented juice samples and the remaining samples. According to Tandon et al. [204], juice contains a high percentage of solids due to water evaporation during increased steam kettle temperature. Where a slight decrease was observed at the end of the storage period, the decrease was for UV treatment from 15 to 13.7 Brix, US treatment from 15 to 13.5 Brix, UV+US from 15 to 14 Brix, for control from 14 to 13 Brix, and the thermal treatment sample from 15 to 14 Brix and fermented sample from 15 to 13.5. However, this decrease was not statistically significant ($p > 0.05$).

Table 4. 13 This table shows the Brix value of samples stored for 4 weeks

Treatment sample	24 hours	1 week	2 weeks	3 weeks	4 weeks
UV	15 ± 1.0^{aA}	15 ± 1.0^{aA}	$14\pm0.8a^{abA}$	12 ± 1.0^{bA}	13.5 ± 1.0^{abA}
US	15 ± 0.5^{aA}	15 ± 0.2^{aA}	14 ± 0.2^{aA}	13.5 ± 1.1^{aA}	13.5 ± 1.3^{aA}
UV+US	15 ± 1.5^{aA}	15 ± 1.0^{aA}	14 ± 1.0^{aA}	$13.5\pm1.^{aA}$	13.5 ± 1.0^{aA}
Control	14 ± 0.9^{Aa}	14 ± 0.6^{aA}	13.5 ± 1.3^{bA}	13 ± 1.0^{cA}	13 ± 0.3^{cA}
Thermal	15 ± 1.0^{Aa}	15 ± 1.0^{aA}	14 ± 0.5^{aA}	13.5 ± 1.2^{aA}	13.5 ± 0.4^{aA}
Fermented Pomegranate	15 ± 1.0^{aA}	15 ± 1.3^{aA}	14 ± 1.0^{aA}	13.5 ± 0.5^{aA}	13.5 ± 1.0^{aA}

Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3)

4.6.6 Turbidity

Changes in turbidity (level, quality) after the treatments performed the UV-C and ultrasound and thermal UV+US and fermented pomegranate juice were determined during 28-days storage, shown in Table 4.14. No significant changes ($P > 0.05$) found between the fermented juice and the unfermented samples. The turbidity levels of UV, US, UV+US, control, thermally and fermented processed beverages were between 4.02±0.1 and 4.00±0.6 NTU, 4.25±0.1 and 4.20±0.7 NTU, 4.75±0.1 and 4.24±0.8 NTU, 4.20±0 and 4.24±01 NTU, 4.85±0.1 and 4.20±0.3 NTU, 4.72±0.1 and 4.24±0.7 NTU. According to the results, turbidity levels were slightly decreased in all samples except for the heat-treated sample. Alternatively, this decrease in level turbidity were observed statistically insignificant in all weeks of storage time, in all samples except for the heat-treated. The decrease in thermally treated juices turbidity value were statistically significant ($p < 0.05$) at the end of storage.

Table 4. 14 Turbidity value of fermented and non-fermented pomegranate juice UV+US, ultraviolet, ultrasound, and through preservation at temperature (5 ± 1°C) for 4 weeks

Treatment sample	24 hours	1 week	2 weeks	3 weeks	4 weeks
UV	4.02 ± 0.1^{aC}	4.02 ± 0.1^{aC}	4.01 ± 0.1^{aC}	4 ± 0.06^{aC}	$4.\pm0.6^{aC}$
US	4.25 ± 0.1^{aB}	4.24 ± 0.1^{aB}	4.22 ± 0.3^{aB}	4.20 ± 0.7^{aB}	4.20 ± 0.7^{aB}
UV+US	4.75 ± 0.1^{aAB}	4.26 ± 0.1^{bAB}	4.24 ± 0.3^{bAB}	4.20 ± 0.3^{bAB}	4.24 ± 0.8^{bAB}
Control	4.2 ± 0.1^{aB}	4.26 ± 0.1^{aB}	4.24 ± 0.3^{aB}	4.21 ± 0.5^{aB}	4.24 ± 01^{aB}
Thermal	4.85 ± 0.1^{aA}	4.75 ± 0.1^{bA}	4.24 ± 0.3^{cA}	4.24 ± 0.3^{cA}	4.20 ± 0.3^{cA}
Fermented pomegranate	4.72 ± 0.1^{aAB}	4.25 ± 0.1^{bAB}	4.21 ± 01^{bAB}	4.20 ± 0.1^{bAB}	4.24 ± 0.7^{bAB}

Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3).

4.6.7 Color Analysis

Color is an important physical property of food, and it provides essential information about food quality. According to the 28-days storage results, the values of pomegranate juices treated with the UV, US, UV+US, thermal treatment, control, and fermented juice shown in Table 4. 15 varied between 3.30±0.1 and 3.45±0.01. The color a * value (redness-greenness), the were no significant changes observed (P> 0.05) for all treatment conditions fermented pomegranate juice samples and the remaining samples.

Pomegranate juice were not significant changes in color a* value affected by the UV-C and ultrasound and thermal treatment, whereas it was observed slightly decreased in color a* value by UV+US and fermented pomegranate juice. However, this decrease in a* value were not significant changes (p >0.05) for fermented and non-fermented pomegranate juices where the result were after 24 h to 28 days for UV, US, UV+US, control, thermal treatment, and fermented pomegranate respectively 3.45±0.00 and 3.40±0.00, 3.44±0.01 and 3.50±0.02, 3.35±0.01 and 3.30±0.1, 3.40±0.01, and 3.36±0.01, 3.45±0.01, and 3.40±0.02, and 3.35±0.01, and 3.30±0.01 as shown in Table 4.15.

Table 4. 15 Color a* value of fermented and non-fermented pomegranate juice UV+US, ultraviolet, ultrasound, and through preservation at temperature (5 ± 1°C) for four weeks

Treatment Sample	24 hours	1 week	2 weeks	3 weeks	4 weeks
UV	3.45 ± 0.0^{aA}	3.45 ± 0.00^{aA}	3.44 ± 0.0^{aA}	3.41 ± 0.0^{aA}	3.40 ± 0.0^{aA}
US	3.44 ± 0.01^{bA}	3.44 ± 0.02^{bA}	3.43 ± 0.01^{bcA}	3.40 ± 0.01^{cA}	3.50 ± 0.02^{aA}
UV+US	3.35 ± 0.01^{aC}	3.35 ± 0.036^{aC}	3.33 ± 0.01^{aC}	3.31 ± 0.01^{cC}	3.30 ± 0.1^{aC}
Control	3.40 ± 0.01^{aB}	3.40 ± 0.01^{aB}	3.39 ± 0.01^{aB}	3.38 ± 0.01^{cB}	3.36 ± 0.01^{dB}
Thermal	3.45 ± 0.01^{aA}	3.43 ± 0.03^{aA}	3.43 ± 0.04^{aA}	3.42 ± 0.01^{cA}	3.40 ± 0.02^{dA}
Fermented pomegranate	3.35 ± 0.01^{aC}	3.35 ± 0.01^{aC}	3.33 ± 0.01^{abC}	3.31 ± 0.01^{bC}	3.30 ± 0.01^{bC}

Mean with superscripts of different small letters in the same columns are significantly different as Tukey's test ($p < 0.05$). The values are expressed as mean ± standard deviation (replications = 3)

4.6.8 Sensory Analysis

The results concerning sensory evaluation observed by 30 consumers for evaluating fermented pomegranate juice and unfermented pomegranate juice in expression of smell, taste, and overall quality preferred are displayed in Table 4.16. No statistically significant difference were discovered excepting for the fourth week of preservation. Consumers preferred fermented pomegranate drink in terms of taste, aroma, and overall quality contrast to unfermented pomegranate berverage at the end of the storage period. Improve the taste of the juice is an exciting discovery in lactic acid fermentation since lactic acid fermentation can increase the taste profile of pomegranate juice [221].

Table 4. 16 Initial sensory evaluation fermented and non-fermented pomegranate juice UV+US, ultraviolet, ultrasound, and (5 ± 1°C) for four weeks

Storage Time	Type	Aroma	Taste	Total Quality
24 hours	Non-fermented	8.6±1.15 [a]	8.6±1 [a]	8.1±1.2 [a]
	Fermented	8.3± 1 [a]	8.6±1.5 [a]	8.2±1.2 [a]
Week 1	Non-fermented	7.6± 0.6[ab]	7.6±1 [ab]	7.7±1.5 [a]
	Fermented	7.3± 1.1 [a]	7.3±1.5 [a]	7.6±1.2 [a]
Week 2	Non-fermented	7.3±0.6 [ab]	7.1± 1 [abc]	7±0.6 [a]
	Fermented	7± 1.1 [a]	7±2.0 [a]	7±1.1 [a]
Week 3	Non-fermented	6.6±0.6 [b]	6.6±0.6 [bc]	6.5±1.5 [a]
	Fermented	6.8± 1 [a]	6.3±1.5 [a]	6.5±0.6 [a]
Week 4	Non-fermented	5.5±1[b]	5.6± 0.6 [c]	5.2± 1.5 [a]
	Fermented	6.5±1.5 [a]	6± 1.0 [a]	6.3±1.0 [a]

Superscript similar letters indicate that there are no statistically significant differences = 0.05 (ANOVA, Tukey's Post Hoc Multiple Comparisons)

The *Lactobacillus plantarum* bacteria can ferment pomegranate juice, and antioxidants and total phenolic content can be obtained better than the non-fermented samples. Whereas our study proved that they could receive a pomegranate drink containing bacteria probiotics through the fermentation of the juice and obtained good results regarding the antioxidant content and the total content of phenol Comparing with non-fermented samples. These results were close to the study previously also prepared on pomegranate juice [218]. All the samples were stored for four weeks, and the samples were evaluated sensory by consumers, who preferred the fermented juice at the end of the storage time over the non-fermented juice in terms of taste, aroma, and general quality.

Besides Mousavi and Razavi [27] in this study, they fermented pomegranate juice with four *Lactobacillus plantarum*, *L. delbruekii*, *L. paracasei, L. acidophilus* bacteria for four weeks. They watched these bacteria's growth every week to determine what kind of bacteria could survive as long as possible. *L. plantarum* and *L. delbruekii* can be survival ab to 2 weeks is better from another's. On the

other hand, there is also a report prepared on pomegranate juice fermentation with bacteria *Lactobacillus plantarum* PU1 as a tool to enhance the antioxidant content. This study proved that fermenting pomegranate juice can increase antioxidants by 40% and can significantly inhibit linoleic acid [222].

Integrated non-thermal processes offer the potential to reduce each method's disadvantages and increase the effectiveness of the method [223]. In the present study, the UV and US treatment were combined, and the PJ were 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 in fruit juice and other fluid pasteurization. Our results show that the UV+US treatment enhanced the pomegranate juice quality parameters compared with the conventional method. Then can use this technique of pasteurization to ferment different juices.

4.7 Conclusion

Consumers expectations for minimal processing, the nutritional value of food, and healthy food are increasing day by day. Therefore, as an alternative to traditional pasteurization processes and their disadvantages, an emphasis has been focused on the use of non-thermal pasteurization recently. 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. Besides, optimization were done to minimize the process of the conditions using these two non-thermal methods. When the results are examined, it will be seen that US pasteurization were more effective than the UV pasteurization technique for microbial results, and both methods provided significant advantages over conventional pasteurization. About the integrated use of UV + US systems, it has been concluded that it has excellent fruit juices pasteurization potential. It has been demonstrated in this study that the antioxidant capacity of juice and the total phenol content can be improved by fermentation of pomegranate juice by the bacteria *Lactobacillus plantarum* pasteurized from combining the two methods UV

+ US to produce healthier juice it has a high level of antioxidants and a total phenol content better than unfermented juice.

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Papers

1- T. A. M. Alabdali, N. C. Icyer, G. U. Ozkaya, and M. Z. Durak, “Effect of stand-alone and combined ultraviolet and ultrasound treatments on physicochemical and microbial characteristics of pomegranate juice,” *Appl. Sci.*, 2020, doi: 10.3390/APP10165458