Food Science and Technology Research, **25** (3), 341–350, 2019

doi: 10.3136/fstr.25.341

http://www.jsfst.or.jp

Original paper

Investigation of the Effects of Non-Thermal, Combined and Thermal Treatments on the Physicochemical Parameters of Pomegranate (*Punica granatum L.*) Juice

Seydi Yıkmış

Department of Nutrition and Dietetics, Tekirdağ Namık Kemal University, Değirmenaltı, Tekirdağ, Turkey

Received November 12, 2018 ; Accepted January 9, 2019

The aim of this study is to investigate the change in microbiological, physicochemical and sensory properties of freshly-squeezed pomegranate juice using ultrasound and/or natamycin as an alternative to pasteurization. Samples were pasteurized (80℃, 2 min), sonicated (60 % amplitude, 26 KHz, 80 W, 5 min), treated with natamycin (12.5 ppm) and stored at 4℃ for one month. Pasteurized pomegranate juice was not significantly changed in terms of pH, Brix, titratable acidity and total phenolic (μg gallic acid equivalent. mL^{-1}) values compared to the treated samples ($p > 0.05$). During storage, ultrasound-processed pomegranate juice retained more than 90 % total monomeric anthocyanins. Microbial values showed a reduction in load at a level acceptable for the general quality parameters. In the sensory analysis, the general evaluation was accepted as ultrasound and/or natamycin. This study emphasizes the potential application of ultrasound and/or natamycin to increase the functional value of pomegranate juice.

Keywords: ultrasound, natamycin, pomegranate juice, non-thermal, sensory analysis

Introduction

Pomegranate (*Punica granatum, L.*) belongs to the family of *Punicacea*, endemic in Turkey, Iran, USA, Middle East, Southeast Asian and Arab countries and is a plant that grows in the Mediterranean (Meerts *et al*., 2009). Pomegranate has fairly good quality because of its sensory and nutritional properties (López-Rubira *et al*., 2005). As polyphenols, it contains flavonoids (anthocyanins), condensed tannins (proanthocyanidins) and hydrolysable tannins (ellagitannins and gallotannins) (Jaiswal *et al*., 2010). It was reported that consumption of pomegranate fruit has many benefits including reduced oxidative stress, effects on LDL, anticancer, antibacterial, and antiviral properties (Qu *et al*., 2010). Research reported pomegranate contains ACE (Angiotensin Converting Enzyme) enzyme which prevents negative effects on blood pressure and was reported to repair damaged blood vessels (Aviram and Dornfeld, 2001). In food processing, pasteurization is a heat treatment which is widely used in the fruit juice industry to obtain a microbiologically safe product. However, anthocyanins and many bioactive compounds are degraded by conventional pasteurization processes (Aadil *et al*., 2013). It has negative effects on sensory and nutritional values. For fresh and natural fruit juices, consumer demand is increasing, but they have limited shelf life. Therefore, there is a need for alternative non-thermal methods that can increase shelf life and maintain nutrition and bioactive properties (Buzrul *et al*., 2008; Patras *et al*., 2009; Plaza *et al*., 2006).

Ultrasound is a non-thermal food preservation method which generally uses 20-40 kHz frequency, in order to inactivate food spoilage microorganisms and enzymes (Ferrante *et al*., 2007; Gómez-López *et al*., 2010). Ultrasound, as a non-thermal technology, with less processing time, higher efficiency and lower energy consumption is an accepted method for fruit juice processing (Charoux *et al*., 2017; Tiwari and Mason, 2012). The effect of ultrasound is primarily due to the effects of free radicals (H and OH), to physical phenomena (acoustic cavitation and the resulting high-speed micro-jets and shock waves) and/or the collapse of the bubbles and the water vapor in them (Gao *et al*., 2014; Herceg *et al*., 2013). Natamycin is a

*To whom correspondence should be addressed. E-mail: syikmis@nku.edu.tr

polyene that serves to bind membrane sterols by disrupting the selectivity of membrane permeability (Elayedath and Barringer, 2002). Natamycin was recognized by the FDA (Food and Drug Administration) as a GRAS (Generally Recognized As Safe) product (Koontz *et al*., 2003). It is approved as a food additive in more than 40 countries (Ollé Resa *et al*., 2016). In molds, the mechanism of action is damaged by binding to the ergosterol in the cell membrane (Abessinio, 2014; Balaguer *et al*., 2014; Jagus *et al*., 2016).

The aim of this study was to investigate and compare the physicochemical, sensory and general microbiological effects of ultrasound and/or natamycin applications in pomegranate juice, which has a high bioactive benefit, as an alternative to pasteurization.

Materials and Methods

Sample preparation Pomegranates were purchased from a local supermarket in Tekirdag (Turkey). Extracting and cleaning operations were performed. Fruit was washed in cold tap water prior to evacuation. Pomegranate fruits were processed using a blender (Waring Blender, USA). Juice was filtered a single layer of muslin cloth to filter out seeds, coarse particles and impurities. The filtered fruit juice samples were stored in sterile glass bottles at 4℃ before being placed in the experiment. Pimalac brand Natamycin (pimaricin) was used. In the study, the UP200St- ultrasound device from Hielscher Ultrasonics (Berlin, Germany) was used. Pasteurization (80℃, 2 minutes), ultrasound (80 W, 26 kHz, 60 amplitude, 5 minutes) and natamycin (12.5 ppm) were applied to the pomegranate juice. The analysis was carried out at 4℃ after 30 days of storage. The ultrasound conditions for this study were selected according to previous reports about ultrasound application in fruit juices (Yıkmış and Aksu, 2018). Classification of sample was C: taze nar suyu, PJ: pasteurized juice, NJ: natamycin juice, UJ: ultrasound juice, UNJ: natamycin+ultrasound juice. All samples were prepared in triplicate.

Microbiological analysis Colony-forming units (CFU) were determined by standard spreading plate methodologies. Dilution of untreated pomegranate juice was done in buffered peptone water and then, PCA (Plate Count Agar-Merck, USA) was used for total aerobic counts (APC). For yeast and mold count (YMC), PDA (Potato Dextrose Agar-Merck, USA) was used. MRS (Man, Rogosa and Sharpe Agar-Merck, USA) containing 50 mg/L tartaric acid was used to inhibit yeast growth and anaerobic incubation was used to determine lactic acid bacteria (LAB). Spreading method was used in the analyzes. Pomegranate juice diluted in 0.1 mL of peptoned juice was added to the agar media. It was calculated as 10 colony in 1 mL as limit of detection. In this method, cells below 10 could not be detected. The plates were incubated at 30℃ for 24-96 hours.

pH, Total soluble solids (Brix) and titratable acidity analysis pH was measured by means of a pH meter. The pH of the samples was analyzed using a Hanna Instruments HI 2002 pH /ORP (Romania) instrument. The number of dissolved solids was determined by the refractometric method. ATAGO brand RX-7000α model (Japan) abdominal refractometer was used for this purpose. Measurements were made at 20℃ and the results were expressed in Brix. The titration acidity was potentiometrically determined by titration of the samples with 0.1 N NaOH (Sigma-Aldrich, USA) solution to pH 8.1. From the sample, 5 mL was taken and 50 mL of distilled water was added and 10 mL of the sample was taken from the filtrate. The results were calculated as g citric acid / L (AOAC, 1995).

Total polyphenols, total flavonoids and antioxidant activity The total amount of phenolic material was modified spectrophotometrically with the Folin-Ciocalteu method. The results are given as gallic acid equivalent (GAE) (Singleton and Rossi, 1965). The total flavonoid content was modified by the aluminum chloride colorimetric analysis method. Total flavonoid content was expressed as epicatechin equivalents (CE) per mL (Zhishen *et al*., 1999). Antioxidant activity was measured by Trolox Equivalent Antioxidant Capacity (TEAC) test (Pellegrini *et al*., 2003). The results were expressed as TEAC in Trolox mmol per liter of sample. The results are found using the standard calibration curve. SP-UV/VIS-300SRB spectrophotometer (Australia) was used for absorbance measurements.

Color analysis Color analysis of the samples was made using the Color Measuring Device PCE-CSM 5 (Germany) and the liquid container. Color L, a, and b color parameters are given (Varela-Santos *et al*., 2012).

Total monomeric anthocyanin and polymeric color analysis The total monomeric anthocyanin content was determined according to the pH differential method. Total anthocyanin contents of pomegranate juices were determined by the pH differential method using two buffer systems: potassium chloride buffer (Sigma-Aldrich, USA), pH 1.0 (0.025 M), and sodium acetate buffer (Sigma-Aldrich, USA), pH 4.5 (0.4 M) (Cemeroğlu, 2010; Giusti and Wrolstad, 2001). The samples were diluted with distilled water according to the predetermined dilution factor as for the determination of total monomeric anthocyanin. For color intensity, bisulfite was calculated by the absorbance value read from the sample which had not had treatment applied. The analysis is based on the definition of "color intensity" and "polymeric color" values (Giusti and Wrolstad, 2001). SP-UV/VIS-300SRB spectrophotometer (Australia) was used for absorbance measurements.

Sensory analysis Sensory analyzes were performed on the first day and last day of treatment. The panelists were asked to evaluate taste, odor, color, texture and overall acceptability of the samples. Fruit juice samples were studied by 10 (5 female, 5 male) panelists. All samples were randomly coded using three-digit letters (ABC, ENO, STR & XYZ). Sensory properties were determined using a 9-point hedonic scale (0-9). Scale scores were excellent, 9; very good 8; good, 7; acceptable, 6; poor (first odorless, tasteless development) <6. Acceptance of sub-points

Table 1. Microbiological results for pomegranate juice with different processes applied

Microbiology	Time (Days)	Treatment				
		C	P	N	U	NU
APC	1	5.65±0.07	ND	4.21±0.34	ND	ND
YMC		5.15±0.03	ND	0.00±0.00	ND	ND
LAB		3.96±0.21	ND	3.93±0.14	ND	ND
APC	30	7.13±0.07	ND	4.21±0.01	ND	ND
YMC		7.15±0.03	ND	ND	ND	ND
LAB		7.31±0.21	ND	3.96±0.74	ND	ND

C: fresh pomegranate juice; ND, not detected, log CFU/mL, all data were means ± SD, n = 3.
C: fresh pomegranate juice, PJ: Pasteurized juice, NJ: Natamycin juice, UJ: Ultrasound juice
UNJ: Natamycin+Ultrasound juice

was accepted as 6. The product was described as unacceptable after initial odor or unpleasantness (Petrou *et al.*, 2012).

Statistical analysis Statistical analyses in the study were conducted with SPSS 20.0 (SPSS Inc., Chicago, U.S.A) and GraphPad Prism 7.0. One-way ANOVA was compared with multiple comparison-Tukey tests. Statistically significant level was determined as $p < 0.05$ in all analyzes. The relationships between the variables were examined using Pearson correlation coefficients. All measurements were done in triplicate and results were expressed as mean value ± standard deviation (SD).

Results and Discussion

Microbiological analysis Pasteurization and sterilization are common thermal methods to reduce microbial load in food products. However, these thermal methods create negative effects on the sensory quality and nutritional value of liquid foods (Lee *et al.*, 2013). Therefore, non-thermal technologies are alternative methods with minimal effects on the characteristics of the liquid food. Table 1 shows the effect of ultrasound treatment on the microorganism content of pomegranate juice. The natamycin sample applied to fresh pomegranate juice showed 4.21 and 3.93 log CFU / mL values for APC and LAB, respectively, which are not found in other applications. Pomegranate juice was successfully passed the general microbiological evaluation. Similar successful results were reported when ultrasound was applied for the destruction of microbial cells (Chemat *et al.*, 2011). Cell disruption can be caused by various factors such as combined physical and chemical mechanisms and the formation of free radicals and hydrogen peroxide, which occur during cavitation (Koda *et al.*, 2009; Oyane *et al.*, 2009)

pH, Total soluble solids (Brix) and titratable acidity The pH, titratable acidity, and Brix values for the pomegranate juice treated with ultrasound and/or natamycin are shown in Table 2. As a result of this study, pomegranate juice which is pasteurized was determined to have no significant change in terms of pH, Brix and titration acidity compared to the treated samples ($p > 0.05$).

Non-thermal applications generally do not have a significant effect on pH, titration acidity and brix values (Charoux *et al.,* 2017). Thermal treatments can vary the pH by varying the different acids present in the juice (Maia *et al.*, 2007). No significant difference was observed in the pH values, titration acidity and Brix in the studies conducted with ultrasound applied to banana juice (sonication treatments; treatment frequency 40 kHz, 50 W, 0-30 min) (Bora *et al.*, 2017), strawberry juice (sonication treatments; 0, 15 and 30 min at 20℃, 25 kHz frequency) (Bhat and Goh, 2017) and carrot-grape juice (sonication treatments; 20 kHz frequency, 70 % amplitude level (525 W power), and pulse duration 5 s on and 5 s off, 5 min at 15℃) (Nadeem *et al.*, 2018).

Total polyphenols, total flavonoids and antioxidant activity The total antioxidant, phenolic and flavonoid values for pomegranate juice treated with ultrasound and/or natamycin are shown in Table 3. As a result of this study, it was determined that there is no significant change in the total phenolic substance value (μg GAE. mL^{-1}) compared to the samples treated with other processes ($p > 0.05$). In the study, the total amount of phenolic substances is in parallel with the literature (Caliskan and Bayazit, 2012; Sepúlveda *et al.*, 2010). Combined processes did not show a statistically significant difference when compared with pasteurized pomegranate juice ($p > 0.05$). Similar results were found in research about phenolic compounds in the blends of apple and cranberry juice treated with combined non-thermal methods (Caminiti *et al.*, 2011). In the ultrasound procedure applied to a carrot-grape juice mixture during storage (1-90 days), total phenolic substance, flavonoid and antioxidant properties were examined for the effect on the total phenolic substances with the amount of storage which decreased during the period (Nadeem *et al.*, 2018). The results are in parallel with the literature. The highest difference was for ultrasound treatment. UJ and UNJ applications were more successful than PJ samples. Reductions in the amount of phenolic compounds

Table 2. The pH, titratable acidity and Brix results for different processes applied to pomegranate juice,

Treatment	Days	pH	TA	Brix
C	1	3.41±0.00[a]	13.23±0.01[a]	16.40±0.00[a]
	10	3.41±0.01[a]	13.31±0.01[b]	16.40±0.00[a]
	20	3.43±0.01[b]	13.41±0.03[c]	16.40±0.01[ab]
	30	3.43±0.01[b]	13.51±0.02[d]	16.41±0.00[b]
PJ	1	3.41±0.01[a]	13.24±0.15[a]	16.42±0.03[a]
	10	3.41±0.01[a]	13.17±0.02[a]	16.45±0.01[a]
	20	3.41±0.01[a]	13.16±0.02[a]	16.44±0.02[a]
	30	3.42±0.01[a]	13.20±0.02[a]	16.45±0.03[a]
NJ	1	3.41±0.01[a]	13.25±0.03[a]	16.42±0.02[a]
	10	3.42±0.01[a]	13.26±0.02[a]	16.42±0.01[a]
	20	3.42±0.01[a]	13.26±0.02[a]	16.45±0.01[b]
	30	3.41±0.01[a]	13.26±0.05[a]	16.43±0.01[ab]
UJ	1	3.41±0.01[a]	13.29±0.06[a]	16.41±0.03[a]
	10	3.42±0.01[a]	13.27±0.02[a]	16.43±0.02[a]
	20	3.42±0.01[a]	13.25±0.03[a]	16.43±0.02[a]
	30	3.41±0.01[a]	13.30±0.05[a]	16.43±0.02[a]
UNJ	1	3.41±0.01[a]	13.28±0.06[a]	16.44±0.01[a]
	10	3.41±0.01[a]	13.26±0.02[a]	16.44±0.01[a]
	20	3.42±0.01[a]	13.30±0.05[a]	16.43±0.02[a]
	30	3.43±0.01[a]	13.51±0.04[b]	16.45±0.02[a]

All data are means ± SD, n = 3. Values with different letters within one column are significantly different ($p < 0.05$). C: fresh pomegranate juice, PJ: Pasteurized juice, NJ: Natamycin juice, UJ: Ultrasound juice UNJ: Natamycin+Ultrasound juice

are thought to be due to the polymerization of phenolic substances or the condensation of phenolic compounds (Klimczak *et al*., 2007; Wang *et al*., 2000).

As a result of the study, a positive correlation was found between all samples in terms of antioxidant (1), phenolic (0.51) and flavonoid material (0.82). As a result of the study, statistically significant differences were found in the amounts of total antioxidants over time and between the applications ($p < 0.05$). UNJ treatment was found to be more successful in maintaining total antioxidant capacity compared to other treatments. Oliverira *et al*. (2018) reported that the results of the non-thermal combination on açai (Euterpe oleracea) juice showed similar results (Oliveira *et al.*, 2018). Reductions in the amounts of antioxidants are thought to be due to the degradation of the antioxidant components by the ultrasound application (Pingret *et al*., 2013).

Color analysis Color is a visual indicator that is used to evaluate the quality of fruit juices during processing and storage and plays an important role in consumer satisfaction (Aadil *et al*., 2013). The values of L (lightness-darkness), a (red or green) and b (yellowness or blueness) for pomegranate juice treated with ultrasound and/or natamycin are shown in Table 4. At the end of the treatment, statistically significant differences were determined in L, a and b values during the storage period between P samples and other applications ($p < 0.05$).

Compared with traditional thermal sterilization methods, non-thermal sterilization is reported to provide better protection for nutrients, flavor and color for juice (Jiménez-Sánchez *et al*., 2017). Adekunte *et al.* (2010) reported that L, a, and b values decreased after the sonication process applied to tomato juice. A decrease in the L value of the fruit juice indicates redness resulting in a darkening color or hydroxymethyl furfural (HMF) (Mohideen *et al*., 2015; Tiwari *et al*., 2009a). It is thought that the differences can be caused by the factors determined by other researchers. Total anthocyanin content is known as the main parameter behind the red color of pomegranate products. As in the study by the researchers, Pearson's correlation coefficient in the study showed that the total anthocyanin content was significantly positive (Cano-Lamadrid *et al*., 2018).

Total monomeric anthocyanin and polymeric color Anthocyanins are quality-determining compounds in red fruits and fruit juices (Weber and Larsen, 2017). Total anthocyanin content (mg cyanidin-3-glucoside / L), color density, polymeric color and percent polymeric color values for pomegranate juice treated with ultrasound and/or natamycin are shown in Table 5. At the end of the treatment, statistically significant differences

Table 3. Results of ABTS (mmol trolox.mL^{-1}), phenolic compounds (µg of acid galic. mL^{-1}) and flavonoid compounds (µg of epicatechin. mL^{-1}) for treated pomegranate juice

Treatment	Days	ABTS (mmol trolox. mL^{-1})	Phenolic compounds (µg of acid galic. mL^{-1})	Flavonoid compounds (µg of epicatechin. mL^{-1})
C	1	25.41±0.65^{a}	1145.0±3.00^{c}	76.86±0.63^{c}
	10	21.51±0.67^{b}	1134.3±5.29^{b}	71.14±0.98^{b}
	20	17.65±0.35^{c}	1124.7±4.16^{b}	68.81±1.14^{ab}
	30	14.69±0.47^{d}	1111.0±3.61^{a}	67.74±0.91^{a}
PJ	1	24.27±0.90^{a}	1140.7±5.10^{a}	76.19±0.34^{a}
	10	21.94±0.71^{b}	1141.3±3.10^{a}	73.82±0.58^{b}
	20	21.06±1.02^{bc}	1132.3±11.2^{a}	73.43±0.27^{b}
	30	19.87±0.26^{c}	1132.3±2.50^{a}	71.77±0.32^{c}
NJ	1	23.23±1.03^{a}	1140.3±4.50^{a}	76.04±0.09^{a}
	10	22.43±0.04^{a}	1138.0±3.60^{a}	73.68±0.32^{b}
	20	21.68±0.51^{ab}	1131.0±7.80^{a}	73.47±0.20^{b}
	30	20.15±0.31^{b}	1131.7±3.10^{a}	71.02±0.12^{c}
UJ	1	20.07±0.16^{a}	1138.7±5.50^{b}	73.74±0.77^{a}
	10	18.99±0.15^{b}	1141.3±3.10^{b}	72.57±0.23^{ab}
	20	18.54±0.21^{c}	1128.7±9.00^{ab}	71.78±0.34^{b}
	30	18.06±0.10^{d}	1123.7±1.50^{a}	68.47±0.31^{c}
UNJ	1	19.66±0.44^{a}	1138.0±3.00^{a}	74.23±1.23^{a}
	10	18.74±0.47^{b}	1139.0±3.60^{a}	72.99±0.33^{ab}
	20	18.44±0.03^{b}	1133.3±9.00^{a}	72.01±0.56^{bc}
	30	17.88±0.22^{b}	1133.0±1.70^{a}	70.02±0.73^{c}

All data are means ± SD, n = 3. Values with different letters within one column are significantly different ($p < 0.05$). C: fresh pomegranate juice, PJ: Pasteurized juice, NJ: Natamycin juice, UJ: Ultrasound juice UNJ: Natamycin+Ultrasound juice

were found between the P samples and other applications during the storage period in total anthocyanin content, color density and percent polymeric color values ($p < 0.05$). There were statistically significant differences in the polymeric color values except for ultrasound ($p > 0.05$). When Pearson correlation coefficient was examined, total anthocyanin content was found to be positively correlated with polymeric color.

Reductions in anthocyanins can occur due to high temperature, pressure, and mechanical effects produced by cavitation and chemical reactions due to the formation of hydroxyl radicals (Aadil *et al*., 2015). Another explanation is that the OH radicals produced by cavitation may essentially disrupt the anthocyanins by opening the rings and due to chalcone formation linked to the temperature rise during sonication (Sadilova *et al*., 2007). In the studies conducted by other researchers, similar changes in red grape (Tiwari *et al*., 2010) and strawberry juice (Tiwari *et al*., 2008) were observed after ultrasound application. In another study, it was found that ultrasound-treated berries and strawberry juice had anthocyanin retention levels of more than 98 %. (Tiwari *et al*., 2009b). At the end of the study, retention rates in storage conditions are as follows; C (83 %), PJ (93 %), NJ (92 %), UJ (92 %) and UNJ (91 %). There was no statistically significant difference between 1, 10 and 20 days after ultrasound treatment. As the ratio of polymeric color increases, it is understood that monomeric anthocyanins are degraded and brown color pigments increase, and the natural color is shortened (Cemeroğlu, 2010). This increase in the amount of polymeric color is thought to be due to the concentration of anthocyanins with other phenolic compounds (Guerrero *et al*., 2001; Monagas *et al*., 2005).

Sensory analysis Sensory analysis values for ultrasound and/or natamycin treated pomegranate juice are shown in Table 6 and Figure 1. At the end of the treatment, statistically significant differences were not detected during the storage period in the smell, mouth feel and clarity values between the P sample and other applications ($p > 0.05$), whereas statistically significant differences were determined for the other properties ($p < 0.05$).

In the ultrasound studies applied to cranberry and apple juice, it was determined that the taste and smell evaluations were affected by the changes in the metal taste or free radicals

Table 4. Results of color (L, a, b) values for treated pomegranate juice

Treatment	Days	L	a	b
C	1	35.35±0.16[a]	17.50±0.07[a]	11.31±0.03[a]
	10	34.10±0.04[b]	17.01±0.24[b]	10.98±0.18[b]
	20	33.77±0.05[c]	15.20±0.07[c]	10.44±0.13[c]
	30	33.38±0.15[d]	14.59±0.06[d]	10.08±0.05[d]
PJ	1	35.23±0.02[a]	17.43±0.02[a]	11.35±0.15[a]
	10	35.17±0.03[ab]	17.18±0.11[a]	11.21±0.08[a]
	20	35.12±0.03[bc]	16.82±0.12[b]	10.82±0.06[b]
	30	35.06±0.02[c]	16.47±0.11[c]	10.70±0.05[b]
NJ	1	35.91±0.16[a]	17.38±0.30[a]	11.27±0.02[a]
	10	35.75±0.01[ab]	16.67±0.08[b]	10.94±0.15[b]
	20	35.62±0.15[bc]	16.47±0.08[b]	10.75±0.01[c]
	30	35.41±0.01[c]	16.26±0.13[b]	10.56±0.02[d]
UJ	1	35.11±0.03[a]	16.45±0.03[a]	10.53±0.00[a]
	10	35.05±0.00[a]	16.04±0.20[ab]	10.38±0.06[b]
	20	34.89±0.06[b]	15.65±0.32[bc]	9.86±0.01[c]
	30	34.71±0.01[c]	15.31±0.03[c]	9.75±0.00[d]
UNJ	1	35.15±0.03[a]	16.43±0.02[a]	10.78±0.04[a]
	10	35.09±0.02[a]	16.30±0.02[a]	10.36±0.03[b]
	20	34.99±0.06[b]	16.26±0.14[a]	10.38±0.06[b]
	30	34.84±0.04[c]	15.88±0.03[b]	10.04±0.17[c]

All data are means ± SD, n = 3. Values with different letters within one column are significantly different ($p < 0.05$). C: fresh pomegranate juice, PJ: Pasteurized juice, NJ: Natamycin juice, UJ: Ultrasound juice UNJ: Natamycin+Ultrasound juice

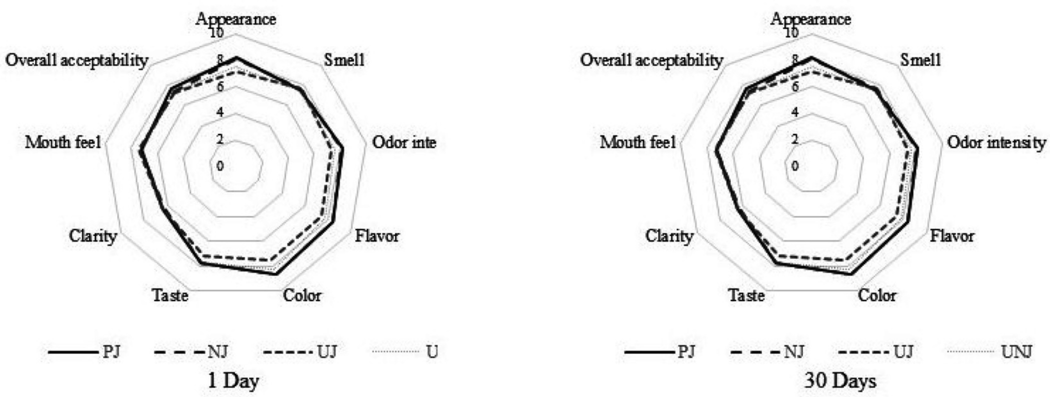

Fig. 1. Results of sensory analysis values chart for treated pomegranate juice

caused by the probes (Caminiti *et al*., 2011; Simunek *et al*., 2013). This was not the case in the study. The researchers evaluated the quality of ultrasound orange juice in terms of standard parameters (taste, taste, smell, mouth sensation etc.). It was found that the sensory properties of ultrasound-treated orange juice were the most acceptable for consumers and were equal to the quality parameters of fresh unprocessed fruit juices (Khandpur and Gogate, 2015). Apple juice and nectar treated with ultrasound were found to have better sensory acceptability than thermally pasteurized juices. The positive effect of the ultrasound process is attributed to the removal of oxygen. Another explanation for the possible changes in the odor and taste of ultrasound-treated fruit juices is the rapid isomerization of the compounds and the emergence of oxidation (Samani *et al*., 2015). As in some studies in the literature, sensory properties were found to be less effective and acceptable than natamycin and ultrasound applications.

Table 5. Results of total anthocyanin content (mg cyanidin-3-glucoside / L), color density, polymeric color and percent polymeric color values for treated pomegranate juice

Treatment	Days	Total anthocyanin content (mg cyanidin-3-glucoside / L)	Color density	Polymeric color	Polymeric color ratio
C	1	119.51±0.25[a]	1.73±0.00[a]	0.63±0.06[a]	36.22±0.33[a]
	10	110.27±0.12[b]	1.65±0.01[b]	0.61±0.00[b]	37.04±0.13[b]
	20	105.30±0.06[c]	1.60±0.01[c]	0.59±0.06[c]	37.08±0.20[ab]
	30	102.07±0.29[d]	1.56±0.01[d]	0.58±0.06[d]	36.89±0.50[ab]
PJ	1	118.07±1.01[a]	1.72±0.01[a]	0.62±0.01[a]	36.17±0.21[a]
	10	117.33±0.17[a]	1.71±0.01[ab]	0.62±0.01[a]	36.19±0.60[a]
	20	114.76±0.62[b]	1.70±0.01[bc]	0.62±0.01[a]	36.35±0.59[a]
	30	110.21±0.97[c]	1.69±0.01[c]	0.63±0.01[a]	37.16±0.5[ab]
NJ	1	117.89±0.88[a]	1.72±0.01[a]	0.63±0.01[a]	36.82±0.18[a]
	10	116.65±1.04[a]	1.71±0.01[b]	0.63±0.02[a]	36.65±0.85[a]
	20	110.42±0.73[b]	1.68±0.01[ab]	0.62±0.01[a]	36.63±0.30[a]
	30	108.55±0.31[b]	1.70±0.02[ab]	0.64±0.01[a]	37.45±0.36[a]
UJ	1	115.24±0.39[a]	1.68±0.01[a]	0.60±0.01[a]	35.59±0.84[b]
	10	113.90±0.30[b]	1.65±0.01[ab]	0.60±0.01[ab]	36.44±0.66[b]
	20	108.90±0.31[c]	1.63±0.01[b]	0.60±0.01[ab]	36.89±0.13[b]
	30	103.76±0.35[d]	1.59±0.02[c]	0.63±0.02[a]	39.71±1.25[a]
UNJ	1	116.18±0.06[a]	1.68±0.02[a]	0.62±0.02[a]	35.79±1.15[b]
	10	114.77±0.49[a]	1.65±0.01[ab]	0.60±0.01[a]	36.49±0.43[b]
	20	109.59±0.58[b]	1.63±0.01[bc]	0.60±0.01[a]	36.53±0.62[b]
	30	105.98±0.81[c]	1.61±0.01[c]	0.63±0.02[a]	39.13±0.87[a]

All data are means ± SD, n = 3. Values with different letters within one column are significantly different ($p < 0.05$). C: fresh pomegranate juice, PJ: Pasteurized juice, NJ: Natamycin juice, UJ: Ultrasound juice UNJ: Natamycin+Ultrasound juice

Conclusion

In this study, the effects of non-thermal ultrasound and natamycin treatment on microbiological, physicochemical and sensory properties were evaluated for pomegranate juice quality. Ultrasound processing was found to provide more successful results for phenolic substances, flavonoids and antioxidant compounds in pomegranate juice. The sensory properties of the treated products were also found to be successful. Ultrasound and natamycin were found to be technologies with the potential to reduce the microbial load in pomegranate juice and to protect bioactive compounds during storage in the refrigerator. In summary, it is concluded that natamycin combined with ultrasound technologies will provide an alternative for the processing of pomegranate juice.

References

Aadil, R.M., Zeng, X.-A., Han, Z., and Sun, D.-W. (2013). Effects of ultrasound treatments on quality of grapefruit juice. *Food Chem*., **141**, 3201–3206.

Aadil, R.M., Zeng, X.-A., Zhang, Z.-H., Wang, M.-S., Han, Z., Jing, H., and Jabbar, S. (2015). Thermosonication: a potential technique that influences the quality of grapefruit juice. *Int. J. Food Sci. Technol*., **50**, 1275–1282.

Abessinio, A. (2014). Use of natural antimicrobials to control spoilage in marinara-type sauce. Mississippi State University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Technology in the Department of Food Science, Nutrition and Health Promotion.

Adekunte, A., Tiwari, B.., Scannell, A., Cullen, P.J., and O'Donnell, C. (2010). Modelling of yeast inactivation in sonicated tomato juice. *Int. J. Food Microbiol*., **137**, 116–120.

AOAC, (1995). Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, DC.

Aviram, M. and Dornfeld, L. (2001). Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. *Atherosclerosis*., **158**, 195–198.

Balaguer, M.P., Fajardo, P., Gartner, H., Gomez-Estaca, J., Gavara, R., Almenar, E., and Hernandez-Munoz, P. (2014). Functional properties and antifungal activity of films based on gliadins containing cinnamaldehyde and natamycin. *Int. J. Food Microbiol*., **173**, 62–71.

Bhat, R. and Goh, K. (2017). Sonication treatment convalesce the

Table 6. Results of sensory analysis values for treated pomegranate juice

Time (Days)	Sensory feature	PJ	NJ	UJ	UNJ
1	Appearance	8.23±0.06[a]	8.13±0.06[a]	7.10±0.10[b]	7.53±0.21[c]
	Smell	7.50±0.30[a]	7.60±0.20[a]	7.63±0.21[a]	7.40±0.10[a]
	Odor intensity	8.17±0.25[a]	8.07±0.23[ab]	7.33±0.06[bc]	7.63±0.06[c]
	Flavor	8.40±0.17[a]	8.37±0.06[a]	7.43±0.06[b]	7.77±0.06[c]
	Color	8.70±0.10[a]	8.70±0.10[a]	7.50±0.10[b]	8.27±0.06[c]
	Taste	7.70±0.36[a]	7.77±0.06[a]	7.17±0.12[b]	7.70±0.17[b]
	Clarity	6.43±0.15[a]	6.30±0.10[a]	6.43±0.21[a]	6.30±0.10[a]
	Mouth feel	7.23±0.15[a]	7.33±0.15[a]	7.43±0.12[a]	7.20±0.10[a]
	Overall acceptability	7.60±0.10[a]	7.30±0.10[ab]	7.27±0.15[ab]	7.33±0.12[b]
30	Appearance	8.18±0.03[b]	8.12±0.08[ab]	7.95±0.05[a]	7.97±0.10[a]
	Smell	7.52±0.28[a]	7.65±0.13[a]	7.57±0.15[a]	7.55±0.23[a]
	Odor intensity	8.07±0.29[a]	7.95±0.18[a]	8.05±0.25[a]	8.02±0.26[a]
	Flavor	8.25±0.23[a]	8.32±0.08[a]	8.25±0.09[a]	8.23±0.18[a]
	Color	8.42±0.28[a]	8.65±0.18[a]	8.38±0.20[a]	8.22±0.08[a]
	Taste	7.65±0.39[a]	7.72±0.08[a]	8.02±0.24[a]	7.62±0.28[a]
	Clarity	6.35±0.23[a]	6.22±0.08[a]	6.38±0.20[a]	6.38±0.24[a]
	Mouth feel	7.12±0.08[a]	7.22±0.08[a]	7.22±0.03[a]	7.15±0.18[a]
	Overall acceptability	7.45±0.28[a]	7.25±0.18[a]	7.23±0.18[a]	7.20±0.05[a]

All data were means ± SD, Means within rows with differing subscripts are significantly different at least $p < 0.05$.
PJ: Pasteurized juice, NJ: Natamycin juice, UJ: Ultrasound juice UNJ: Natamycin+Ultrasound juice

overall quality of hand-pressed strawberry juice. *Food Chem*., **215**, 470–476.

Bora, S.J., Handique, J., and Sit, N. (2017). Effect of ultrasound and enzymatic pre-treatment on yield and properties of banana juice. *Ultrason. Sonochem*., **37**, 445–451.

Buzrul, S., Alpas, H., Largeteau, A., and Demazeau, G. (2008). Inactivation of Escherichia coli and Listeria innocua in kiwifruit and pineapple juices by high hydrostatic pressure. *Int. J. Food Microbiol*., **124**, 275–278.

Caliskan, O. and Bayazit, S. (2012). Phytochemical and antioxidant attributes of autochthonous Turkish pomegranates. *Sci. Hortic. (Amsterdam)*., **147**, 81–88.

Caminiti, I.M., Noci, F., Muñoz, A., Whyte, P., Morgan, D.J., Cronin, D.A., and Lyng, J.G. (2011). Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. *Food Chem*., **124**, 1387–1392.

Cano-Lamadrid, M., Hernández, F., Nowicka, P., Carbonell-Barrachina, A.A., and Wojdyło, A. (2018). Formulation and storage effects on pomegranate smoothie phenolic composition, antioxidant capacity and color. *LWT - Food Sci*., **96**, 322–328.

Cemeroğlu, B. (2010). Gıda Analizleri, 2nd ed. *Nobel Yayıncılık*, Ankara.

Charoux, C.M.G., O'Donnell, C.P., and Tiwari, B.K. (2017). Ultrasound Processing and Food Quality, in: Bermúdez-Aguirre, D. (Ed.), Ultrasound: Advances for Food Processing and Preservation. *Elsevier*, 215–235.

Chemat, F., Zill-e-Huma, and Khan, M.K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrason. Sonochem*., **18**, 813–835.

Elayedath, S. and Barringer, S.A. (2002). Electrostatic powder coating of shredded cheese with antimycotic and anticaking agents. *Innov. Food Sci. Emerg. Technol*., **3**, 385–390.

Ferrante, S., Guerrero, S., and Alzamorat, S.M. (2007). Combined use of ultrasound and natural antimicrobials to inactivate Listeria monocytogenes in orange juice. *J. Food Prot*., **70**, 1850–1856.

Gao, S., Hemar, Y., Ashokkumar, M., Paturel, S., and Lewis, G.D. (2014). Inactivation of bacteria and yeast using high-frequency ultrasound treatment. *Water Res*., **60**, 93–104.

Giusti, M.M. and Wrolstad, R.E. (2001). Anthocyanins. Characterization and measurement of anthocyanins by UV-visible spectroscopy, in: Wrolstad, R.E. Schwartz, S.J. (Ed.), In Current Protocos in Food Analytical Chemistry. John Wiley & Sons, New York, U.S.A, pp. 1–13.

Gómez-López, V.M., Orsolani, L., Martínez-Yépez, A., and Tapia, M.S. (2010). Microbiological and sensory quality of sonicated calcium-added orange juice. *LWT - Food Sci. Technol*., **43**, 808–813.

Guerrero, S., López-Malo, A., and Alzamora, S. (2001). Effect of ultrasound on the survival of Saccharomyces cerevisiae: influence of temperature, pH and amplitude. *Innov. Food Sci. Emerg. Technol*., **2**, 31–39.

Herceg, Z., Markov, K., Sobata, B.Š., Jambrak, R.A., Vukušić, T., and Kaliterna, J. (2013). Effect of High Intensity Ultrasound Treatment on the Growth of Food Spoilage Bacteria. *Food Technol. Biotechnol*., **51**, 352–359.

Jagus, R.J., Gerschenson, L.N., and Ollé Resa, C.P. (2016). Combinational Approaches for Antimicrobial Packaging: Natamycin and Nisin, in: Antimicrobial Food Packaging. pp. 599–608.

Jaiswal, V., DerMarderosian, A., and Porter, J.R. (2010). Anthocyanins and polyphenol oxidase from dried arils of pomegranate (Punica granatum L.). *Food Chem*., **118**, 11–16.

Jiménez-Sánchez, C., Lozano-Sánchez, J., Segura-Carretero, A., and Fernández-Gutiérrez, A. (2017). Alternatives to conventional thermal treatments in fruit-juice processing. Part 2: Effect on composition, phytochemical content, and physicochemical, rheological, and organoleptic properties of fruit juices. *Crit. Rev. Food Sci. Nutr*., **57**, 637–652.

Khandpur, P. and Gogate, P.R. (2015). Effect of novel ultrasound based processing on the nutrition quality of different fruit and vegetable juices. *Ultrason. Sonochem*., **27**, 125–136.

Klimczak, I., Małecka, M., Szlachta, M., and Gliszczyńska-Świgło, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *J. Food Compos. Anal*., **20**, 313–322.

Koda, S., Miyamoto, M., Toma, M., Matsuoka, T., and Maebayashi, M. (2009). Inactivation of Escherichia coli and Streptococcus mutans by ultrasound at 500 kHz. *Ultrason. Sonochem*., **16**, 655–659.

Koontz, J.L., Marcy, J.E., Barbeau, W.E., and Duncan, S.E. (2003). Stability of Natamycin and Its Cyclodextrin Inclusion Complexes in Aqueous Solution. *J. Agric. Food Chem*., **51**, 7111–7114.

Lee, H., Kim, H., Cadwallader, K.R., Feng, H., and Martin, S.E. (2013). Sonication in combination with heat and low pressure as an alternative pasteurization treatment – Effect on Escherichia coli K12 inactivation and quality of apple cider. *Ultrason. Sonochem*., **20**, 1131–1138.

López-Rubira, V., Conesa, A., Allende, A., and Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biol. Technol*., **37**, 174–185.

Maia, G.A., Sousa, P.H.M. de, Santos, G.M. dos, Silva, D.S. da, Fernandes, A.G., and Prado, G.M. do, (2007). Effect of the processing on some components of acerola juice. *Ciência e Tecnol. Aliment*., **27**, 130–134.

Meerts, I.A.T.M., Verspeek-Rip, C.M., Buskens, C.A.F., Keizer, H.G., Bassaganya-Riera, J., Jouni, Z.E., van Huygevoort, A.H.B.M., van Otterdijk, F.M., and van de Waart, E.J. (2009). Toxicological evaluation of pomegranate seed oil. *Food Chem. Toxicol*., **47**, 1085–1092.

Mohideen, F.W., Solval, K.M., Li, J., Zhang, J., Chouljenko, A., Chotiko, A., Prudente, A.D., Bankston, J.D., and Sathivel, S. (2015). Effect of continuous ultra-sonication on microbial counts and physico-chemical properties of blueberry (Vaccinium corymbosum) juice. *LWT - Food Sci. Technol*., **60**, 563–570.

Monagas, M., Bartoleme, B., and Gomez-Cordoves, C. (2005). Updated Knowledge About the Presence of Phenolic Compounds in Wine. *Crit. Rev. Food Sci. Nutr*., **45**, 85–118.

Nadeem, M., Ubaid, N., Qureshi, T.M., Munir, M., and Mehmood, A. (2018). Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. *Ultrason. Sonochem*., **45**, 1–6.

Oliveira, A.F.A., Mar, J.M., Santos, S.F., da Silva Júnior, J.L., Kluczkovski, A.M., Bakry, A.M., Bezerra, J. de A., Nunomura, R. de C.S., Sanches, E.A., and Campelo, P.H. (2018). Non-thermal combined treatments in the processing of açai (Euterpe oleracea) juice. *Food Chem*., **265**, 57–63.

Ollé Resa, C.P., Gerschenson, L.N., and Jagus, R.J. (2016). Starch edible film supporting natamycin and nisin for improving microbiological stability of refrigerated argentinian Port Salut cheese. *Food Control*, **59**, 737–742.

Oyane, I., Takeda, T., Oda, Y., Sakata, T., Furuta, M., Okitsu, K., Maeda, Y., and Nishimura, R. (2009). Comparison between the effects of ultrasound and γ-rays on the inactivation of Saccharomyces cerevisiae: Analyses of cell membrane permeability and DNA or RNA synthesis by flow cytometry. *Ultrason. Sonochem*., **16**, 532–536.

Patras, A., Brunton, N.P., Da Pieve, S., and Butler, F., (2009). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innov. Food Sci. Emerg. Technol*., **10**, 308–313.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., and Brighenti, F. (2003). Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different In Vitro Assays. *J. Nutr*., **133**, 2812–2819.

Petrou, S., Tsiraki, M., Giatrakou, V., and Savvaidis, I.N. (2012). Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *Int. J. Food Microbiol*., **156**, 264–271.

Pingret, D., Fabiano-Tixier, A.-S., and Chemat, F. (2013). Degradation during application of ultrasound in food processing: A review. *Food Control*, **31**, 593–606.

Plaza, L., Sánchez-Moreno, C., Elez-Martínez, P., de Ancos, B., Martín-Belloso, O., and Cano, M.P. (2006). Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. *Eur. Food Res. Technol*., **223**, 487–493.

Qu, W., Pan, Z., and Ma, H. (2010). Extraction modeling and activities of antioxidants from pomegranate marc. *J. Food Eng*., **99**, 16–23.

Samani, B.H., Khoshtaghaza, M.H., Lorigooini, Z., Minaei, S., and Zareiforoush, H. (2015). Analysis of the combinative effect of ultrasound and microwave power on Saccharomyces cerevisiae in orange juice processing. *Innov. Food Sci. Emerg. Technol*., **32**, 110–115.

Sepúlveda, E., Sáenz, C., Peña, Á.G., Robert, P., Bartolomé, B., and Gómez-Cordovés, C. (2010). Influence of the genotype on the anthocyanin composition, antioxidant capacity and color of Chilean pomegranate (Punica granatum L.) juices. *Chil. J. Agric. Res*., **70**, 50–57.

Simunek, M., Jambrak, A., Petrovic, M., Juretic, H., Major, N., Herceg, Z., Hruskar, M., and Vukusic, T. (2013). Aroma Profile and Sensory Properties of Ultrasound-Treated Apple Juice and Nectar. *Food Technol. Biotechnol*., **51**, 101–111.

Singleton, V. and Rossi, A., (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent, *Am. J. Enol. Vitic*., **16**, 155-158

Tiwari, B.K. and Mason, T.J. (2012). Ultrasound Processing of Fluid Foods, in: Novel Thermal and Non-Thermal Technologies for Fluid Foods. Elsevier, pp. 135–165.

Tiwari, B.K., O'Donnell, C.P., and Cullen, P.J. (2009a). Effect of sonication on retention of anthocyanins in blackberry juice. *J. Food Eng*., **93**, 166–171.

Tiwari, B.K., O'Donnell, C.P., and Cullen, P.J. (2009b). Effect of non thermal processing technologies on the anthocyanin content of fruit juices. *Trends Food Sci. Technol*., **20**, 137–145.

Tiwari, B.K., O'Donnell, C.P., Patras, A., and Cullen, P.J. (2008). Anthocyanin and Ascorbic Acid Degradation in Sonicated Strawberry Juice. *J. Agric. Food Chem.*, **56**, 10071–10077.

Tiwari, B.K., Patras, A., Brunton, N., Cullen, P.J., and O'Donnell, C.P. (2010). Effect of ultrasound processing on anthocyanins and color of red grape juice. *Ultrason. Sonochem*., **17**, 598–604.

Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J.E., Pérez-Won, M., Briones-Labarca, V., and Morales-Castro, J. (2012). Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innov. Food Sci. Emerg. Technol*., **13**, 13–22.

Wang, L., Kim, D., and Lee, C. (2000). Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. *J. Agric. Food Chem*., **48**, 4227–32.

Weber, F. and Larsen, L.R. (2017). Influence of fruit juice processing on anthocyanin stability. *Food Res. Int*., **100**, 354–365.

Yıkmış, S. (2018). Inactivation of Botrytis cinerea and Investigations on Changes in Some Quality Parameters with Ultrasonication and Natamycin Combination in Grape Juice. Inst. Heal. Sci. Dep. Hyg. Technol. Dr. Thesis. Istanbul University.

Zhishen, J., Mengcheng, T., and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*., **64**, 555–559.