Effect of Sonication Treatments on Freshly Squeezed Mandarin Juice Quality Parameters

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In this study, sonication treatment at 35 kHz for 0, 5, 15, 30, and 60 minutes (at 20°C) and thermal pasteurization at 95°C for 15 seconds were applied to freshly squeezed mandarin juice to determine the effects on physicochemical properties, bioactive compounds, and other quality parameters. The changes for pH, titratable acidity, water activity, browning index, total flavonoids, antioxidant activity, ascorbic acid contents, color, and cloud (in sensory analysis) were insignificant. However, increases in viscosity, ash, turbidity, and color values and decreases in enzyme activities were observed after the sonication treatment. Overall, for 15 minutes treatment, significant enhancement in total phenolics and ascorbic acid content was observed. Results suggest that 15 minutes of sonication treatment could contribute to the improvement of the quality of mandarin juice and therefore, it is possible to employ this treatment in mandarin juice processing successfully.

Introduction

Citrus, which is one of the most cultivated fruits worldwide is appreciated by most consumers. Citrus fruits and their derived products are beneficial to human health. From the past to the present, they have been consumed due to their nutritional and antioxidant properties and their ability to prevent disease.[1] Citrus fruits can be abundantly found and cultivated worldwide among various natural fruits. Mandarins are one of the most popular citrus fruits. Mandarins are rich in organic acid and phenolic compound content. The taste characteristics and organoleptic quality of these fruits are largely affected by their content and concentrations.[2]

Conventional thermal treatments such as pasteurization can cause to impair the nutritive value, sensorial quality, and functional properties of foods. Regarding the unwanted effects of heat on food preservation and sanitation, alternative procedures such as ultrasound have been employed.[3] Ultrasound processing called sonication is an emerging technology due to its cheap, simple, reliable, environmentally friendly, and effective application to achieve microbial decontamination. Ultrasound has already been considered an alternative to heat

treatment in fruit juice processing without adversely affecting their nutritional quality and health benefits.[4]

Ultrasounds are produced by the conversion of electrical energy into mechanical one by using piezoelectric materials.[5] Ultrasound treatment is classified as low-intensity ultrasound and high-intensity ultrasound, where frequency ranges are 5–10 MHz and 20–100 kHz respectively.[6] The energy transmitted to the food through ultrasound processing can be expressed as power ultrasound (W), ultrasound intensity (W/cm^2), acoustic energy density (W/mL), or cavitational intensity.[7] Patil *et al*.[8] reported that sonication results in microbubble formation in the food system that implodes which is called cavitation. These microbubbles collapse violently in the succeeding compression cycles of a propagated sonic wave.[4]

According to the literature review, there are harldy any exhaustive study about the influence of sonication and pasteurization treatments on the freshly squeezed mandarin juice quality. The evaluated quality criteria include physicochemical properties such as pH titratable acidity, total soluble solid, viscosity, water activity, moisture, ash, browning index, cloud assessment, turbidity, color, HMF, total phenolic content, total carotenoids, total flavonoid content, ascorbic acid content, antioxidant activity, PPO activity, PME activity, invert sugar, sensory analyses.

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Materials and Methods

Preparation of Mandarin Juice Samples and Pasteurized Mandarin Juices Production

Fremont (F) (*Citrus reticulata*, hybrid of Clementine × Ponkan) mandarins were obtained from a local market that is located in Adana, Turkey. Following their arrival at the laboratory, mandarins were washed very well under tap water to remove adhering dirt and impurities. After that, with the help of a pre-sterilized knife, they were cut into two halves and pressed using a pre-sterilized hand presser (the percentage yield of mandarins was 43.90%). Seeds and coarse pulp were removed by passing the mandarin juices through 1 mm stainless steel sieves. Pasteurization and sonication treatment were immediately applied to the mandarin juices (Fig. 1). The pasteurization process was applied at 95°C for 15 seconds, where process conditions were determined by preliminary experiments. Pasteurized mandarin juices were stored in amber color bottles at 4°C for further analysis. All analyses and treatments were done three times.

Sonication Treatment

Sonication treatment was applied right after the juice extraction. Each mandarin juice sample was poured into pre-sterilized glass beakers and samples were labeled into four independent batches (0, 5, 15, 30, and 60 minutes) and sonication treatment was applied. The sonication treatment conditions were adjusted to 35 kHz frequency at 560 watts (at 20°C) with regular monitoring of the homogenous sound field distribution. The treatment was carried out with a "Bandelin Sonorex Ultrasonic Bath" (Model RK103H, Germany). After treatment time, mandarin juices were stored in sterile beakers wrapped with aluminum foil (to provide darkness to avoid interference by light).

Sonicated mandarin juice, pasteurized juice, and control (freshly squeezed or no treatment) samples produced in the laboratory were subjected to the following analyses:

Physicochemical Analysis

The pH and TSS (Total Suspended Solids) analyses were made by WTW pH-meter (Weilheim, Germany) and Abbe refractometer (J.P. Selecta, WYA-25,
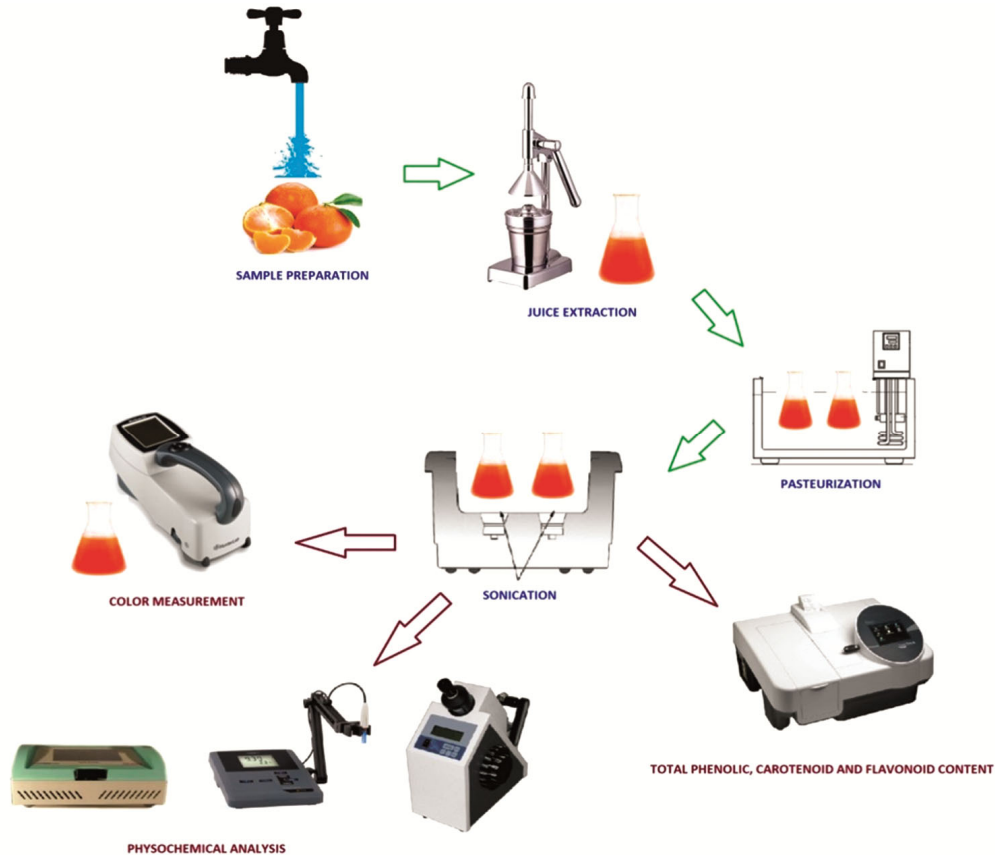

Fig. 1 — Representations of treatments on mandarin juices

Spain), respectively. Titratable acidity was made by an endpoint titration with 0.1 N NaOH where the endpoint was pH 8.1. The results were calculated as citric acid and were expressed as g/100mL.[9] The water activity and viscosity values were measured with a water activity device (Novasina Labmaster Water Activity Meter, Switzerland) and a Fungilab Expert viscometer (Model L, Sant Feliu de Llobregat, Barcelona) respectively. The viscosity measures were taken at 100 rpm with spindle TL7 and TL5 and the cP values were determined. For moisture content analysis, the 2 g example in the drying vessel, is based on the principle that the sample is held at 65°C under vacuum conditions at 10 bar until constant weighing is reached. At the end of this period, the sample containers were weighed to room temperature in a desiccator. The amount of dry matter in the samples was calculated as % according to the following formula[10]:

$$\text{Moisture } \% = \frac{(G_3 - G_1) \times 100}{G_2 - G_1}$$

In percentage ash analysis, about 2 g of each sample was placed in porcelain containers and ignited and incinerated in the ash furnace at about 550°C for 8 hours. The total ash was expressed as percent ash on a dry basis: %Ash = (Weight of residue/Sample Weight)*100.[11]

Determination of Browning Index, Cloud Assessment, and Turbidity

In Teflon tubes, 5 mL sample and 5 mL ethyl alcohol (95%) were mixed, and then centrifugation was applied at 2576g, 10 min, at 4°C. After that, the supernatant was filtered by using a 0.45 μm Teflon membrane filter. The absorbance of the filtered supernatant was measured in a spectrophotometer (Biochrom, Libra S60, B, England) at 420 nm.[12] Furthermore, for cloudiness assessment, 10 mL of the control and ultrasonic-treated juice samples were centrifuged at 2063g at 25°C for 10 min and the supernatant was collected and measured at the absorbance of 660 nm afterward (UV–vis spectrophotometer, Biochrom, Libra S60, B, England).[13] Turbidity in all samples was measured using a spectrophotometer at 610 nm wavelength. The respective turbidity was calculated by the following equation (distilled water was taken as a reference)[13]:

Transmittance (T) = 100 × (100 − Abs)
Turbidity = 100 − T

where, Abs = absorbance and T = transmittance at a wavelength of 610 nm

Color Measurement

Color measurement (CIE L*, a*, b*) was performed with HunterLab Spectrophotometer (HunterLab miniscan EZ, USA). Approximately 50 mL sample was placed into a 20 mm (diameter) Glass Optical Cell. The results were obtained according to the CIELAB color system. In this procedure, L* describes lightness (0-100 : black-white), a* indicates the red/green value ((+) values indicate red; (−) values indicate green) and b* the yellow/blue value ((+) values indicate yellow; (−) values indicate blue). The hue angle (o) shows a specific blue, red, green, or yellow color or a combination of these colors. Chroma shows the intensity of a color. Also, the formulas given below were utilized for Hue* and C* calculations:

$$Hue^* = \arctan\left(\frac{b^*}{a^*}\right)$$
$$C = \sqrt{(a^*)^2 + (b^*)^2}$$

Determination of HMF Content

The 5-hydroxymethylfurfural (HMF) content was determined quantitatively with the method based on the colorimetric reaction between barbituric acid, p-toluidine, and HMF, forming a red color complex; which is described by Cemeroglu.[14]

Total Phenolic Contents

Total phenolic contents were determined using the spectrophotometric method. A standard curve was obtained by standard gallic acid solutions with different concentrations. For this, 0.5 mL sample, 2.5 mL Folin-Ciocalteu's reagent (10%), and 2.5 mL $NaHCO_3$ (7.5%) were added. Incubation was carried out at 45°C for 45 min in a water bath. After, the gallic acid equivalent in samples was calculated according to absorbances at 765 nm (Biochrom, Libra S60, B, England).[9]

Total flavonoid contents

The aluminum chloride colorimetric method was used(catechol as a standard). Initially, 1 mL sample was diluted sixfold then mixed with 0.3 mL $NaNO_2$ (5%), and incubated for 5 min. Subsequently, 0.6 mL of $AlCl_3.6H_2O$ (10%) solution was added to the samples and incubated for 5 min. The mixture was completed to 10 mL by adding 2 mL of 1 M NaOH

solution and double-distilled water. After 15 min incubation, the absorbance measurements were taken by UV-VIS spectrophotometer at 510 nm (Biochrom, Libra S60, B, England). Total flavonoid content values were calculated as mg/L catechol.[9]

Total Carotenoid Content

The previously described slightly modified method of Lee and Castle[15] was used for total carotenoid determination. Sample of 5 mL was mixed with 10 mL hexane solution (hexane/methanol/acetone, 50/25/25, v/v with 0.1% BHT) and centrifuged at 2576g for 10 min at 4°C. The absorbances of the supernatant phase were measured (450 nm) using a spectrophotometer. Total carotenoid content was calculated by using the β-carotene extinction coefficient ($E_{1/2} = 2505$).

Ascorbic Acid Content

According to Ucan Türkmen and Mercimek Takci[9], the ascorbic acid content was determined when 2,6-diclorophenol-indophenol was used as a color reagent by measuring the absorbance at 518 nm. The ascorbic acid content was determined with the standard curve prepared with L-ascorbic acid.

DPPH Radical Scavenging Activity

The antioxidant capacities of all samples were determined by DPPH (2,2-diphenyl 1-picrylhydrazyl) radical scavenging activity. In brief, 3.9 mL DPPH solution (0.025 g/L in methanol) was added to 100 μL of the sample and the mixture was stirred with a vortex device. Afterward, the incubation was carried out at room temperature in the dark for 120 min. The amount of remaining DPPH was determined bymeasuring the absorbance at 515 nm in a spectrophotometer(Biochrom, Libra S60, B, England). The DPPH inhibition was calculated as a percent with the formula I% = [($A_{blank} - A_{sample}$)/A_{blank}] × 100.[9]

Invert Sugar Content

0.5 mL samples were taken and 1 mL DNS reagent (3,5-dinitrosalicylic 10.6 g, NaOH 19.8 g for 1416 mL distilled water) was added. This mixture was boiled at 90°C for 5 minutes. The reaction was terminated by the addition of 1 mL of 1 M Rochelle's (Seignette) salt. Thereafter, the mixture was diluted by adding 2 mL distilled water, and the absorbance was spectrophotometrically measured at 595 nm to estimate reducing sugars. Glucose was used as standard reducing sugar in this analysis and the results were expressed as 1 μmol glucose that was liberated per min.[16]

Polyphenoloxidase Enzyme Activity

To 2.9 mL of 10 M catechol substrate (prepared in 0.1 M KH_2PO_4 buffer (pH 6.8) at room temperature) 0.1 mL of juice sample was added. The change in absorbance was monitored for 5 min. One unit of PPO enzyme activity was described as the amount of enzyme that changes the absorbance by 0.001 per minute.[17]

Determination of PME Activity

A modified method of Kimball[18] was used in the PME (Pectin methylesterase) activity determination of the samples. To determine the PME activity, 10 mL of orange juice was added to 20 mL of 1% pectin-salt substrate (0.1M NaCl) and incubated at 30°C. The pH of the solution was adjusted to 7.0 with 2.0 N NaOH and then readjusted to pH 7.7 by using 0.05 N NaOH. 0.10 mL of 0.05 N NaOH was added after the pH reached 7.7. Time was measured (t') until the pH regained the value of 7.7 after the addition of 0.10 mL of 0.05 N NaOH. PME activity (%) was calculated with the formulas given below, where, $A0$ is the PME activity of the untreated sample which was determined immediately after processing to avoid the effects of storage time, (t') is time in min, and At is PME activity after the treatments. PME activity (A) was calculated as:

$$A = \frac{(0.05N - NaOH).(0.10\,ml - NaOH)}{(t)(10 - ml\,sample)}$$

$$Residual\,PME\,Activity\,(\%) = \left(\frac{A_t}{A_0}\right)100$$

Sensory Analysis

Quantitative descriptive analysis (QDA) was used for sensory evaluation After the treatments, the samples were subjected to QDA by ten trained panelists. Samples labeled with randomly selected 3-digit code numbers. To eliminate the residual taste between samples, water was provided to the panelists. The evaluated attributes were: color, sweetness, acidity off-odor, and general impression. Unstructured line scales (10 cm) anchored at the ends with terms related to minimum and maximum intensities were used to evaluate each attribute.[19]

Statistical Analysis

For statistical analyses, analysis of variance (ANOVA) and Duncan's multiple comparison tests were applied and the software SPSS 22.0 (Windows)

(SPSS Inc., Chicago, IL, USA) was used. All analyses were triplicated.

Results and Discussion

Physicochemical Analyzes (pH, Total soluble solids, Titratable acidity, Water activity, Moisture, Ash, and Viscosity)

The obtained values for physicochemical analyzes of treated and control samples are shown in Table 1. A real indication of quality,pH values for control and pasteurization (past) samples were 3.75 and 3.73, respectively ($p>0.05$). These values decreased with time in ultrasound treatments.Fruit juice is considered highly acidic if the pH is <4.6, and this type of juice usually does not require drastic thermal treatments. So also, acidity in fruit juices can be correlated to flavor.[13]

Titratable acidity for the control sample was initially 0.83 g/100mL but these values decreased with pasteurization and ultrasound treatments (0.81 g/100mL). The pH ranged from 3.59 to 3.75 and titratable acidity ranged between 0.81 and 0.84 g/100 mL in all samples ($p>0.05$). The results on insignificant changes in the pH and titratable acidity are in agreement with the observations on sonicated orange, grapefruit, mango, and Kasturi lime juices.[20–23]

The Brix increased to about 5.22% with pasteurization treatments. This value was initially 13.4 and decreased to 12.3, 13.1, 12.9 and 12.9 after 5, 15, 30, and 60 min sonication treatment ($p < 0.05$). These results based on Brix were similar to the investigation on sonicated Kasturi lime (*Citrus microcarpa*) juices.[23] Tomadoni et al.[19] reported that sonication and traditional treatment did not cause any changes in the brix and titratable acidity values of

strawberry juice. Similarly, Santhirasegaram et al.[22] stated to observe no statistical differences between 15, 30, and 60 min ultrasound treatment at 40 kHz frequency in titratable acidity and brix values and traditional methods of 30 and 60 s at 90ºC on mango juice.

The water activity (a_w) ranged from 0.987 (control) to 0.992 in 60 min sonicated samples, respectively. This activity was generally increased depending onsonication time in ultrasound treatments, but it was non-significant statistically ($p > 0.05$). Raso and Barbosa-Cánovas[24] said that the higher water activity expresses the existence of free water in the medium. They asserted that because of the destruction of water molecules produced by the sound waves, the generation of free radicals and the lethality of the microorganisms rise.

The percentage moisture value was 86.85% in the control sample. It decreased to 86.45, 86.59, 86.06, and 86.20% after sonication treatments (5, 15, 30, and 60 min), respectively ($p < 0.05$). This value was detected as 85.22% in the past sample.

The ash content is a measure of the total mineral amount in food. Whereas the mineral content is the amount of specific inorganic components such as Ca, Na, K, and Cl. The ash values ranged from 0.68 to 1.03% in untreated and treated samples ($p < 0.05$). The lowest ash % value was obtained after 60 min sonication (0.89%).

Similarly, the viscosity value increased with pasteurization and sonication treatments. This value in the control sample was 1.79 cP and increased to 1.98 and 1.89, 1.93, 1.91, and 1.92 after pasteurization and sonication treatments (5, 15, 30, and 60 min),

Table 1 — Effects of treatments on physicochemical analyzes in mandarin juices

Treatments	pH	Total Soluble Solid (Brix)	Titratable Acidity (g/100 mL)	Water Activity (a_w)	Moisture (%)	Ash (%)	Viscosity (cP)
Control (untreated or 0 min)	3.75 ± 0.02[a]	13.4 ± 0.36[b]	0.83 ± 0.03[a]	0.987 ± 0.00[b]	86.85 ± 0.02[a]	0.68 ± 0.03[b]	1.79 ± 0.08[c]
Past	3.73 ± 0.09[a]	14.1 ± 0.26[a]	0.82 ± 0.02[a]	0.988 ± 0.00[b]	85.22 ± 0.07[d]	0.96 ± 0.06[a]	1.98 ± 0.03[a]
5 min	3.60 ± 0.13[a]	12.3 ± 0.06[c]	0.84 ± 0.02[a]	0.988 ± 0.00[b]	86.45 ± 0.04[b]	1.01 ± 0.19[a]	1.89 ± 0.02[b]
15 min	3.59 ± 0.19[a]	13.1 ± 0.67[b]	0.82 ± 0.02[a]	0.987 ± 0.00[b]	86.59 ± 0.01[b]	1.03 ± 0.07[a]	1.93 ± 0.03[ab]
30 min	3.59 ± 0.12[a]	12.9 ± 0.15[bc]	0.81 ± 0.05[a]	0.988 ± 0.00[ab]	86.06 ± 0.01[c]	0.99 ± 0.01[a]	1.91 ± 0.03[ab]
60 min	3.61 ± 0.08[a]	12.9 ± 0.45[bc]	0.81 ± 0.01[a]	0.992 ± 0.00[a]	86.20±0.25[c]	0.89±0.15[a]	1.92 ± 0.02[ab]

Values followed by different superscripted letters within the same column are significantly different from each other (p < 0.05)

respectively. This difference between treatments was significant ($p < 0.05$). Our results are similarto the observations on sonicated carrot juice reported by Zou and Jiang.[25] Aadil *et al*.[21] said that the increase or decrease in viscosity of sonicated grapefruit juices depends on the intensity of ultrasound and the structure of food molecules. For the sonicated carrot (*Daucus carota*) juice, similar changes were notified by Adiamo *et al*.[26] Abid *et al*.[27], pointed to an increase in the viscosity of the sonicated apple juice as compared to the untreated sample. This increase is directly connected to the ultrasound methods that bring about the extraction of bound form of macromolecules and raise their concentration in the colloidal system and become more viscous.[28]

Browning Index, Cloud Assessment, Turbidity, PPO, and PME Activity

In Table 2, results on the browning index are presented. The browning index value was increased from 0.073 up to 0.088, 0.080, and 0.076 in the past, 30 and 60 min sonicated mandarin juice samples, respectively. The difference between treatments was insignificant($p > 0.05$). Aadil *et al*. [21] asserted that an increase in the browning index may be connected to the deterioration of color pigments, reaction, and diversity of natural pigments. Santhirasegaram *et al*.[22] reported a significant increase in the browning index of Chokanan mango (*Mangifera indica L.*) juice processed by sonication and heat. Tiwari *et al*.[20] detected an increase in the browning of orange juice with the sonication method. Conversely, no significant impact was determined in the browning index of sonicated carrot juice with pulp in other study.[26]

In addition, outcomes on cloud assessment unclosed a significant increase after ultrasound treatment (5, 15, 30, and 60 min sonicated samples) (Table 2).

The highest value was the 60 min sonicated sample (1.394 abs.) while the lowest value was the pasteurization sample (0.510 abs.) ($p < 0.05$). Cloud value remarks the color and existence of particles in fruit juices that are connected to the particles that constitute a mixture of pectin, protein, lipids, cellulose, and some other minor components.[21] An increase of values in sonicated orange, grape, and strawberry fruit juices is reported in different stydies[13,20,21] supporting our observations. The cause of the increase may be connected to the ultrasound method that splitted larger molecules into smaller ones because of the high-pressure gradient applied by cavitation and expansion of the surface area, which advanced the cloud value in the juices.[29]

As seen in Table 2, turbidity values of samples ranged from 76.64 (untreated samples) to 96.47% (60 min sonicated sample). Cloud assessment values, turbidity values increased with increasing ultrasound time ($p < 0.05$). The increase could be clarified by the mechanical stress emerging from the cavitational collapse of bubbles, which gives rise to the deterioration of large macromolecules and particles in the juice.[22] Bhat and Goh[13], asserted to range from 97.96% (in control) up to 98.05% and 98.03% in 15 and 30 min sonicated samples, respectively.

Polyphenol oxidase (PPO) (oxidizes o-diphenols into o-quinones) activityvalues reduced with increasing ultrasound time and this reduction was found significant ($p < 0.05$).The decrease was uncovered with pasteurization treatment (3.30 U/L). The lowest values were determined as 3.13, 3.13, and 3.65 U/L in 15, 30, and 60 min sonicated samples(Table 2). Fonteles *et al*.[30] reported adecrease in PPO activity withsonication in melon juice. Researchers showed areduction in PPO activity after sonication treatments.[13] Researchers reported increasingthe inactivation of PPO by increasing treatment time.[27] Cheng *et al*.[31] asserted that sonication treatments did enhance PPO activity; contrarily they decrease its activity. They found that PPO activity in the control sample of guava juices was initially 10.1 U, after sonication, this value increased to 18.0 U.

Table 2 — Effects of treatments on the browning index, cloud assessment, turbidity values, PPO, and PME activity in mandarin juices

Treatments	Browning index (abs.)	Cloud assessment (abs.)	Turbidity (%)	PPO Activity (U/L)	PME Activity (sec)
Control (untreated or 0 min)	0.073 ± 0.02^a	0.587 ± 0.08^b	76.64 ± 1.31^c	4.17 ± 0.00^b	100 ± 0.00^a
Past	0.088 ± 0.01^a	0.510 ± 0.01^b	77.06 ± 3.19^c	3.30 ± 0.01^d	44 ± 13.08^c
5 min	0.064 ± 0.02^a	0.615 ± 0.05^b	82.81 ± 4.03^{bc}	4.87 ± 0.00^a	47.67 ± 3.51^c
15 min	0.072 ± 0.01^a	0.808 ± 0.16^b	89.11 ± 4.03^b	3.13 ± 0.01^e	43.00 ± 2.65^c
30 min	0.080 ± 0.01^a	0.787 ± 0.22^b	87.63 ± 6.52^b	3.13 ± 0.01^e	67.67 ± 0.58^b
60 min	0.076 ± 0.02^a	1.394 ± 0.29^a	96.47 ± 2.57^a	3.65 ± 0.01^c	49.33 ± 13.32^c

Values followed by different superscripted letters within the same column are significantly different from each other (p < 0.05)

Results on PME (catalyze de-esterification of the methoxyl group of pectin) activity showed a decrease after pasteurization and sonication treatments and this reduction was significant at all treatments ($p < 0.05$). This value was detected as 44 (sec) after pasteurization while determined as 49.33 (sec) after 60 min sonication treatment. Enzyme activity decreases as a result of sonication treatments vary on temperature and time.[27] Conversely, Wu *et al*.[32] notified that ultrasound treatments were beneficial to get tomato juice with a low residual activity of PME.

Color (L*, a*, b*, Hue and Chroma), HMF and İnvert Sugar

The color of mandarin juices is an essential feature for consumer senses as a quality indicator. Moreover, color changes in fruit juice may be a sign of inaccurate treatment or microbial growth.[23] As seen in Table 3, all color values of untreated and treated samples were statistically significant ($p < 0.05$). The L* value, defined as the brightness of a color, in the control sample, was 21.06, which detected insignificant changes after pasteurization and sonication treatments (26.18, 17.87, 23.55, 23.50 and 25.57 in past, 5, 15, 30 and 60 min sonicated samples, respectively). As application time increases these values generally were increased. a* value for the control sample was initially 9.93 but this value increased with pasteurization (10.45) while decreased with ultrasound treatments (7.57, 7,63, 8,36 and 7.74 in 5, 15, 30 and 60 min sonicated samples, respectively). b* value ranged from 24.11 to 36.37 in untreated and sonicated mandarin juice samples. This value increased based on treatment time. The hue angle (o) value ranged from 68.97 (past) to 77.90 (60 min sonicated sample). Chroma value ranged from 25.27 (5 min sonicated sample) to 37.19 (60 min

sonicated sample). The highest L* and a* values were found after pasteurization and 60 min sonication treatment while the lowest b*, hue, and chroma showed after 5 min sonication treatment.

Aadil *et al*.[21] noticedaslight increase in L* and a slight decrease in a* and b* values during sonication Santhirasegaram *et al*.[22] detected to observe an increase in lightness (L*), and a decrease in redness (+a*) and yellowness (+b*) after sonication. They asserted that these increases and decreases may be expressed with the accumulation of undecided particles in the juice, partly precipitated. Cheng *et al*.[31] determined that a decrease in L*, and an increase in a* and b* values were monitored in guava juices after sonication. Bhat *et al*.[23] observed that the sonicated juices for 60 min demonstrated the minimum value for L* and a* values and the maximum value for b* value. Changing the color of a fruit juice by sonication may be because of cavitation which triggered some physical, chemical, and biological reactions.[33] L*, a*, and b* values gradually increased with treatment time.[25]

In Table 3, results on HMF values are presented.The beginning HMF value in the control sample was 2.70 mg/L and this value increased to 6.05, 5.67, 5.72, 5.94, and 6.37 after pasteurization and sonication treatments (5, 15, 30, and 60 min), respectively. The lowest HMF value was in 5 min sonicated sample and the highest value was in 60 min sonicated sample ($p < 0.05$).

Invert sugar values ranged from 36.80 (30 min) to 59.31 μmol/mL (15 min) as seen in Table 3. Relating to invert sugar, significant decreases and increases were determined in juices with ultrasound when compared with control samples ($p < 0.05$). It can be

Table 3 — Effects of treatments on color (L*, a*, b*, Hue and Chroma), HMF and invert sugar values in mandarin juices

Treatments	L*	a*	b*	Hue	Chroma	HMF (mg/L)	Invert Sugar (μmol/mL)
Control (untreated or 0 min)	21.06 ± 0.69bc	9.93 ± 0.17a	29.11 ± 0.65bc	71.16 ± 0.09cd	30.75 ± 0.67b	2.70 ± 0.41b	41.13 ± 1.89b
Past	26.18 ± 2.19a	10.45 ± 0.37a	27.65 ± 4.24bc	68.97 ± 3.73d	29.60 ± 3.83bc	6.05 ± 0.09a	45.1 ± 2.82b
5 min	17.87 ± 2.49c	7.57 ± 0.18b	24.11 ± 1.41c	72.55 ± 0.60bc	25.27 ± 1.40c	5.67 ± 0.56a	42.14 ± 1.95b
15 min	23.55 ± 2.81ab	7.63 ± 0.81b	31.55 ± 3.07ab	76.40 ± 1.01a	32.46 ± 3.13ab	5.72 ± 0.09a	59.31 ± 9.76a
30 min	23.50 ± 1.43ab	8.36 ± 0.50b	31.99 ± 0.84ab	75.36 ± 0.51ab	33.07 ± 0.93ab	5.94 ± 0.94a	36.80 ± 1.56b
60 min	25.57 ± 3.39a	7.74 ± 0.07b	36.37 ± 4.10a	77.90 ± 1.18a	37.19 ± 4.02a	6.37 ± 0.41a	40.26 ± 1.15b

Values followed by different superscripted letters within the same column are significantly different from each other (p < 0.05)

Table 4 — Effects of treatments on total phenolics, total flavonoids, total carotenoids, ascorbic acid, antioxidant activity, and total pectin content values in mandarin juices

Treatments	Total phenolics (mg/L)	Total flavonoids (mg/L)	Total carotenoids (mg/L)	Ascorbic acid (mg/L)	DPPH (% Inhibition)
Control (untreated or 0 min)	425.26 ± 24.23^e	105.12 ± 4.43^a	200.77 ± 2.31^a	58.40 ± 2.31^{ab}	87.48 ± 1.17^a
Past	489.79 ± 24.64^d	105.55 ± 7.01^a	135.09 ± 18.41^c	56.03 ± 2.99^b	86.79 ± 0.98^a
5 min	560.72 ± 20.72^c	103.87 ± 0.37^a	115.50 ± 14.70^c	61.60 ± 3.70^a	87.84 ± 0.96^a
15 min	760.82 ± 24.74^a	103.56 ± 2.44^a	193.11 ± 13.16^a	61.86 ± 1.73^a	86.89 ± 0.31^a
30 min	732.99 ± 27.84^a	105.16 ± 5.48^a	168.09 ± 16.93^b	58.59 ± 2.58^{ab}	86.49 ± 0.31^a
60 min	633.51 ± 31.44^b	108.78 ± 2.73^a	114.97 ± 1.78^c	61.15 ± 0.84^a	87.23 ± 0.68^a

Values followed by different superscripted letters within the same column are significantly different from each other (p < 0.05)

said that increased the reaction yield of invertase with ultrasound.

Total Phenolics, Total Flavonoids, Total Carotenoids, Ascorbic Acid Contents, DPPH Free Radical Scavenging Activity

Total phenolics in sonicated mandarin juices recorded a significant increase compared to the control and after pasteurization ($p < 0.05$) (Table 4). This increase in total phenolics was from 425.26 mg/L up to 560.72, 760.82, 732.99, and 633.51 mg/L in sonicated samples (5, 15, 30, and 60 min, respectively). Significant increase in total phenolics content with ultrasound treatments that are determined in apple, mango, orange, grapefruit, strawberry, Kasturi lime, and purple cactus pears juice supported our observation.[4,13,21–23,27,34] Abid et al. [27] said to observe an increase in polyphenolic compounds in sonicated apple juices as compared to untreated samples. Moreover, they asserted that the increase in these polyphenolic compounds in sonicated apple juice might be ascribed to the increase in extraction efficacy by sonication treatment causing disruption of cell walls and ultimately liberation of bound polyphenolic compounds. Furthermore, these increases can also be explained by the addition of hydroxyl radicals to the aromatic ring of phenolic compounds during sonication.[23]

Total flavonoid values in sonicated juices showed insignificant increases and decreases compared to the control and past samples ($p > 0.05$). These values ranged from 103.56 (15 min sonicated sample) to 108.78 (60 min sonicated sample) mg/L (Table 4). The total flavonoids and flavonols in sonicated Kasturi lime juices were recorded to increase.[23]

Total carotenoids in mandarin juices indicated a significant decrease (control sample, 200.77 mg/L) after pasteurization and sonication treatments ($p < 0.05$). The highest value was recorded as 193.11 mg/L in 15 min sonicated sample while the lowest value was determined as 114.97 mg/L in 60 min sonicated sample (Table 4). This could be attributed to the carotenoid isomerization due to the high shearing effect after 60 min of sonication and agrees with Santhirasegaram et al. [22] Lee and Coates[35] observed that thermal treatment induces geometric isomerization of carotenoids, resulting in a significant loss of carotenoids in orange juice. In addition, they said that heat causes instability of the conjugated double-bond system of carotenoids, resulting in oxidation.

Ascorbic acid which has been linked with protection against several types of cancers is a major antioxidant.[36] Almost all citrus fruits such as lemon, grapefruit, orange, and mandarin are rich sources of ascorbic acid. As seen in Table 4, ascorbic acid contents of untreated and treated mandarin juices ranged from 56.03 (past sample) to 61.86 (15 min) mg/L ($p > 0.05$). It can be said that the preservation of ascorbic acid is better in 15 min sonicated sample. According to Zafra-Rojas et al.[4], the content of ascorbic acid in almost all the treatments was similar to the control sample. The lowest value was recorded as 56.03 after pasteurization treatment. It can be said that the loss of ascorbic acid might be due to its heat-sensitive characteristic. In the same way, Tiwari et al.[20] determined a higher ascorbic acid content in untreated and sonicated juices than in pasteurized orange juices. This higher ascorbic acid content may be attributed to the removal of dissolved oxygen which is essential for ascorbic acid degradation during cavitation.[31]

Regarding percent inhibition of DPPH radical scavenging activity, insignificant decreases were calculated in sonicated juice compared to untreated samples ($p > 0.05$) (see Table 4). The highest values were recorded as 87.84% in 5 min sonicated samples while the lowest values were detected as 86.49% in 30 min sonicated samples. Bhat and Goh[13], recorded that total antioxidant activity values

Table 5 — Effects of treatments on sensory analysis in mandarin juices

Treatments	Color	Cloud	Taste	Odor	General Impression
Control (untreated or 0 min)	7.45 ± 2.10^a	6.10 ± 3.13^a	7.60 ± 3.05^a	8.30 ± 1.62^a	8.90 ± 1.79^a
Past	7.15 ± 1.94^a	6.20 ± 2.71^a	7.70 ± 2.14^a	7.35 ± 1.99^a	8.20 ± 1.32^{ab}
5 min	7.80 ± 2.51^a	7.25 ± 2.41^a	6.40 ± 2.50^a	6.15 ± 3.11^{ab}	$7.60 \pm .35^b$
15 min	6.90 ± 2.57^a	7.20 ± 2.18^a	7.75 ± 2.63^a	$7.35 \pm .20^a$	7.90 ± 1.60^{ab}
30 min	6.25 ± 2.44^{ab}	7.10 ± 2.04^a	5.30 ± 2.65^a	7.35 ± 2.12^a	7.20 ± 1.03^b
60 min	4.70 ± 2.24^b	7.20 ± 2.43^a	2.60 ± 2.45^b	4.25 ± 3.16^b	5.22 ± 0.44^c

Values followed by different superscripted letters within the same column are significantly different from each other ($p < 0.05$)

increased with sonication and this increase can be related increase in ascorbic acids, anthocyanins, and total phenolics content. Bhat *et al.*[23] indicated increase in DPPH inhibition by ultrasound treatment in Kasturi lime juice. Improvements in DPPH free radical scavenging activity of the sonicated apple juice is reported by Abid *et al.*[27] Chokanan mango (*Mangifera indica L.*) juice samples prepared by thermal treatment demonstrated insignificant changes in the percentage of DPPH inhibition. In the sonicated mango juice samples, the percentage of DPPH inhibition increased compared to the control.[22] It can be said that cavitation predicated by sonication can increase the extraction of antioxidant compounds.

Sensory Evaluation of Untreated and Treated Mandarin Juices

Sensory characteristics which contribute to the consumer approval or refusal of the product are of high importance. To assess the effect of sonication treatment on the organoleptic characteristics of the mandarin juice compared to both control and pasteurized juices, sensory analyses were done (Table 5). Control, pasteurized and sonicated mandarin juice samples were offered to 10 participants. They evaluated the samples in terms of color, cloud, taste, odor, and general impression. Color and cloud values were detected to be statistically insignificant ($p > 0.05$) while taste, odor, and general impression values were significant ($p < 0.05$). Color values ranged from 4.70 (60 min) to 7.80 (5 min). Cloud values ranged from 6.20 (past) to 7.25 (5 min). Odor values ranged from 4.25 (60 min) to 8.30 (control). Taste values are 2.60 (60 min) to 7.75 (15 min). General impression values ranged from 5.22 (60 min) and 8.90 (control). Concerning general impression values, the most favorite sample was 15 min sonicated samples after control and past samples. It can be said that the increased reason of taste scores could be connected to the destruction of the cell because of sonication methods that extract sugars from intracellular spaces to the liquid.[27] Tomadoni *et al.*[19] observed that sonication treatments significantly affected acidity and sweetness scores while color and of-odor scores did not affect by the treatments.

Conclusions

In the present study, the effects of sonication and pasteurization treatments were worked on some quality parameters of freshly squeezed mandarin juice. It was detected that changes occurring in pH, titratable acidity, water activity, browning index, total flavonoids, antioxidant activity, ascorbic acid, and color values after treatment were not much significant. The obtained results revealed that 15 minutes of sonication treatment is sufficient to improve the quality of mandarin juice. Significant increases in the level of ascorbic acid and total phenolics have been seen after a 15-minute treatment. It may be deduced to be a convenient method for the enhancement in total phenolics and ascorbic acid content of mandarin juice. This study creates new avenues for research into comparisons between ultrasonic bath and thermal treatment in mandarin juice.

Conflict of interest

The authors declare no conflict of statement.

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