Food and Nutrition Sciences, 2023, 14, 300-314
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

# Effect of Ultrasound and Thermal Pasteurization on Physicochemical Properties and Antioxidant Activity of Juice Extracted from Ripe and Overripe Pineapple

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**How to cite this paper:** Alam, A., Biswas, M., Ahmed, T., Zahid, M.A., Alam, M., Hasan, M.K., Biswas, B. and Zaman, R. (2023) Effect of Ultrasound and Thermal Pasteurization on Physicochemical Properties and Antioxidant Activity of Juice Extracted from Ripe and Overripe Pineapple. *Food and Nutrition Sciences*, **14**, 300-314.
https://doi.org/10.4236/fns.2023.144020

**Received:** February 23, 2023
**Accepted:** April 11, 2023
**Published:** April 14, 2023



## Abstract

It has become necessary to assess how food processing methods affect qualitative qualities due to the increased consumer awareness of the health benefits of various nutrients in food. In the current study, the effects of ultrasound treatment (37˚C, 5 min), pasteurization (90˚C, 5 min), and their combination on quality parameters, including pH, total soluble solids (TSSs), titratable acidity (TA), color values (L*, a*, b*), ascorbic acid (AA), total phenolic content (TPC), and antioxidant activity (DPPH), of pineapple juice from ripe and overripe pineapples were assessed. Color values (L*), ascorbic acid (AA), total phenolic content (TPC), and DPPH radical scavenging activity in all juice samples that were sonicated alone and in combination with pasteurization improved significantly ($p < 0.05$), but the TSS and color value (a* and b*) decreased in comparison to the control. Whereas pH and acidity did not change significantly ($p < 0.05$). Pasteurization significantly ($p < 0.05$) reduces these attributes, but sonicated samples significantly ($p < 0.05$) improved numerous quality parameters and antioxidant activity, notably in ripe juice. Overall, pasteurization degraded these liquids but sonication, either alone or in combination with it, was advantageous for preserving their quality by retaining nutrients.

## Keywords

Pineapple Juice, Ultrasound, Pasteurization, Phenolic Compounds, Antioxidant Activity

## 1. Introduction

Pineapple is a significant tropical fruit (*Ananas comosus* L.), which is a family member of *Bromeliaceae* and is primarily consumed in the form of processed foods like juices. The sweet and tart flavor of pineapple juice is preferred by consumers. This distinct flavor of pineapple is produced by a combination of phenolic chemicals, furanone, amino acids, and amines [1]. Antioxidant components in this product, including carotenoids, bromelain, phenolic compounds, ascorbic acid, and flavonoids, have been linked to its protective properties. Bromelain (BRM), an active proteolytic enzyme found in pineapple, is employed in medications to treat thrombosis, inflammatory diseases, and malignant tumors [2]. Generally, pineapple contains 80% - 85% water and 10% - 14% sugar content and it is highly accepted all over the world for its high nutritional content. It is commonly consumed fresh, as well as having its meat and juice employed in the manufacture of various products in agro-processing companies. Due to its health-promoting benefits and customer demand, however, agro-processing companies have begun using both ripe and overripe pineapple as a functional ingredient in fruit juice, fruit salad, pies, cakes, ice cream, yogurt, and other products. For such industrial activities, it is essential to have knowledge of the changes in pineapple properties throughout storage [3]. Besides microbial contamination, enzymatic breakdown is a common cause of spoilage in pineapple goods, which consumers often reject [4]. In order to reduce this loss, different processing methods or treatments should be applied to pineapple juice as well as pineapple flesh.

Thermal pasteurization is frequently used to destroy dangerous germs and some enzymes in the juice. Due to the use of high heat in the pasteurization process, the nutritional and sensory qualities of pasteurized juice may be slightly affected [5]. Significant causes of quality deterioration have been found as non-enzymatic browning reactions and pigment loss, both of which can be triggered by heat treatment [6]. Consequently, there is a significant demand for innovative treatments that have less of an effect on the nutritional value and general quality of food. Sonication (ultrasound) treatment, a novel technique regarded as cost-effective, straightforward, dependable, and environmentally friendly, has been investigated for usage in a variety of applications, including fruit juice processing [7] [8] [9]. Recent research on the effect of ultrasonication on cantaloupe melon [10], kasturi lime juice [7], apple juice [11], and orange juice [12] has demonstrated that it is a suitable method of juice processing, capable of preventing more losses in valuable nutrients and reducing the microbial load than thermal processing. The primary benefits of ultrasonic processing are a higher cloud value, lower energy consumption, and less nutritional and taste losses [13]. Thermosonication can substitute thermal processing and is more efficient than ultrasound treatment or pasteurization alone. Several juices, including tomato juice, are available [14], star fruit juice [15], pear juice [16], and apple juice [17] showing an increase in fruit juice quality following thermosonication.

There is a scarcity of knowledge on ripe and overripe pineapple fruit juice, despite the several issued papers on the effects of ultrasonography, pasteurization, and their combination on fruit juices. The objective of this study was to examine the effect of ultrasound, pasteurization, and their combination on the physicochemical properties, phytochemical properties, and antioxidant activity of pineapple juice at different maturity stages.

## 2. Materials and Methods

### 2.1. Sample Preparation

Ripe and overripe pineapples were bought from a local market in Jashore, Bangladesh. According to Shamsudin *et al.* [18] and Ramallo and Mascheroni [19], fruits were gently divided into two separate groups based on their maturity (ripe and overripe). Under flowing water, all pineapples were washed. The fruits were then peeled and pressed mechanically using an electrical juice separator (BL25C46, Guangdong Midea Electric Appliances Co., Ltd., Foshan, China). In order to extract the juice and remove the solid particles, the flesh was squeezed and passed through a double layer of filter cloth. The juices were then kept at −18˚C in a (400 ± 2 ml) glass jar for later analysis. Frozen fruit juices that were ripe or overripe were collected, defrosted, and given time to warm up before being pasteurized and sterilized using ultrasound. After that, the juices are divided into four categories (Group 1: Fresh raw pineapple juice without any treatment; Group 2: Juice treated with pasteurization; Group 3: Juice treated with ultrasonication, Group 4: Juice treated with combined pasteurization and ultrasonication treatment).

### 2.2. Experimental Design

The following treatments were applied to the various groups. Control groups consisted of fresh pineapple juice that had not been treated. Juices were processed by pasteurization and ultrasonication treatments described by Bavisetty and Venkatachalam [20] with slight modification. For pasteurization process, juices were transported into sterilized glass jars (400 ± 2 ml). Juices were then pasteurized (≈90˚C) using a temperature-controlled water bath with a basin made of stainless steel. The water bath (Human Lab Instrument Co., Ltd., Korea) included thermostatic control, temperature resistance up to 120˚C, an operational temperature range of 25˚C - 100˚C with a precision of ±1˚C, and 220 V/50 Hz electrical supply after the pasteurization was finished, samples were gathered and quickly cooled to ambient temperature (25˚C ± 1˚C). The samples were then prepared for ultrasonic therapy.

The ultrasound therapy was carried out using an ultrasound bath (Branson Model 2510E-DHT ultrasonic cleaner, CT 06813, USA), operating at a frequency of 40 kHz with a maximum input power of 185 W (the internal dimensions: 16 × 15.5 × 14.5 inch) for 5 minutes. To prevent any interaction from light with the samples, all treatments were carried out in complete darkness. A sample of untreated juice was similarly preserved and used as a comparison standard (non-sonicated). Stainless steel container-mounted piezoelectric sandwich-type transducers convert high-frequency electricity into ultrasonic waves of the required frequency. Pineapple juice was placed in glass jars and placed in the center of the ultrasonic cleaning solution. During the ultrasonic treatment, the temperature of the demineralized water was 37˚C ± 1˚C (**Figure 1**).

Pineapple juices were also treated with combined treatment of pasteurization and ultrasonication. Same parameters were also set here for both pasteurization and ultrasonication as described earlier. The processed juice was stored at −18˚C until further analysis. For each treatment, three samples were created. The experiment was carried out three times.

## 2.3. Physicochemical Properties

### 2.3.1. Estimation of pH, Total Soluble Solid (˚Brix), Titratable Acidity (TA)

A pH meter was utilized to determine the sample solution's pH (FE20-FiveEasy, Mettler Toledo, Inc., Greifensee, Switzerland). Before analysis, the pH meter was calibrated with buffer solutions of pH 7.0 and pH 4.0. The pH was then determined at 20˚C by adding 25 mL of the sample solution to a 100 mL beaker and stirring (20 rpm) continuously with a mechanical stirrer (Ms-H380-Pro., DLAB Scientific, China).

Using a digital refractometer, total soluble solid (TSS) in ˚Brix was measured (HI 96801 refractometer, China). Each evaluation was completed at 27˚C, and the refractometer's prism was meticulously cleaned with demineralized water [21].

20 mL of pineapple juice and 80 mL of demineralized water were added to a 250 mL beaker in order to determine the total acidity of the juice (TA). Then, this mixture was titrated to a 1% alcoholic phenolphthalein (one gram in one

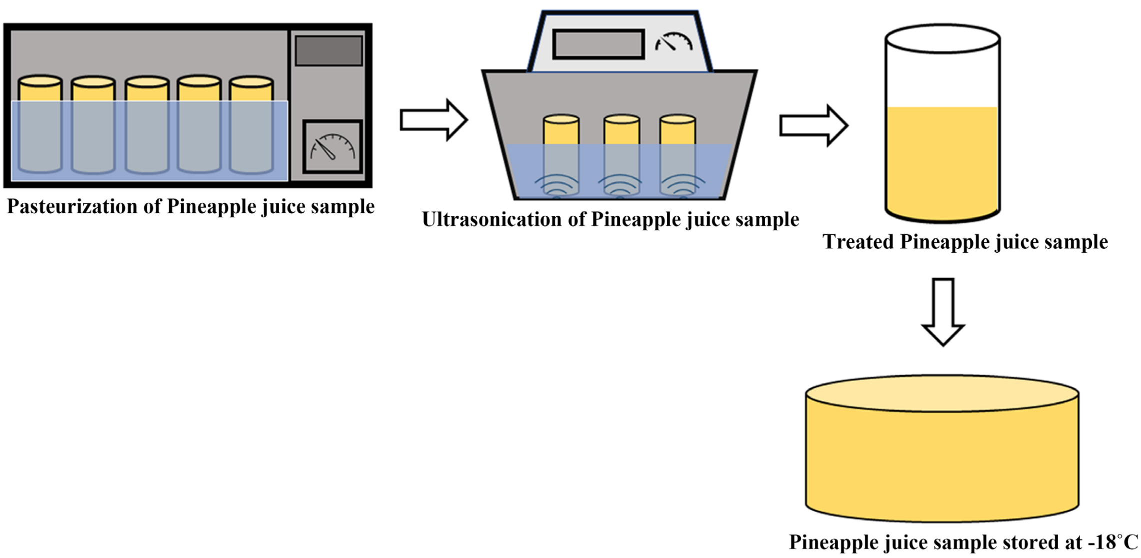


**Figure 1.** Schematic diagram of pasteurization and ultrasonication process of ripe and overripe pineapple juice.

hundred milliliters of pure alcohol) standard solution to 0.1 N NaOH (four grams of NaOH dissolved in one thousand milliliters of demineralized water), to the point of a light pink color. Redd *et al.* [22] estimated titratable acidity (TA) by converting the volume of NaOH to grams of citric acid per 100 milliliters of pineapple juice. Titratable acidity (TA) was computed using Formula (1).

$$\text{Titratable acidity} = \frac{V_{\text{titre}} \times 0.067 \times 0.1\text{N NaOH} \times 100}{m} \quad (1)$$

where, $V_{\text{titre}}$ is titre volume of NaOH, and $m$ is volume of Pineapple juice (ml). Each analysis was conducted in triplicate.

#### 2.3.2. Estimation of Ascorbic Acid (Vitamin C)

The quantity of ascorbic acid in pineapple juice was determined using a validated analytical method [23]. 2,6-dichlorophenolindophenol measures the amount of ascorbic acid present and the redox indicator's capacity to be scavenged by it (coloring). Using the sample's ascorbic acid as a marker, the concentration was measured while 2% oxalic acid was present ($C_2H_2O_4$). 20 mL of the substance and 100 mL of oxalic acid were homogenized three times for a total of 10 seconds. The substance was homogenized, then filtered before being titrated with dye solution. Additionally, the following Equation (2) was used for calculation.

$$\text{Ascorbic acid}\left(\text{mg/100ml juice}\right) = \frac{\text{titre} \times \text{concentration} \times 100}{\text{extract used for estimation} \times \text{volume of sample used for estimation}} \quad (2)$$

### 2.4. Physical Properties

#### Estimation of Color Value

By using a Minolta colorimeter (CR-400, Minolta Camera Co., Ltd., Osaka, Japan) and the Hunter Lab color system, the color of pineapple juice that had been treated was determined. Before the measurement, the colorimeter was calibrated using demineralized water (L = 100, a = 0, b = 0). The color value was determined using a glass cuvette ($3.5 \times 4 \times 1.5$ cm$^3$) containing processed pineapple juice. Three Hunter parameters, "L*" (lightness), "a*" (redness and greenness), and "b*" (yellowness and blueness), were measured, and L*, a*, and b* values were used to determine total color differences.

### 2.5. Phytochemical Properties

#### 2.5.1. Total Phenolic Content (TPC)

Alothman *et al.* [24] devised a method for determining the TPC of pineapple juice. 200 µL of diluted pineapple juice and 1 mL of Folin-Ciocalteu's phenol reagent are mixed. The FC phenol reagent was tenfold diluted with pure water before to use. After holding the pineapple juice—Folin-Ciocalteu's phenol reagent combination at 25˚C ± 0.5˚C for 5 minutes, 800 µL of 7.5% $Na_2CO_3$ was added. The prepared solution was properly mixed and left at ambient temperature for two hours. The absorbance was then measured at 765 nm using a UV-Visible spectrophotometer (UV-1600PC, Shanghai, China).The results were expressed as mg GAE/100 mL of juice using the mean of three measurements.

#### 2.5.2. DPPH Free Radical Scavenging Activity

A method described by Sadiq *et al.* [25] was slightly modified to test the free radical scavenging capacity of controlled and treated pineapple juice. A known quantity of the juice (2 mL) was added with 2 mL of a DPPH solution (0.2 mM in ethanol) before being incubated at ambient temperature (25˚C ± 1˚C) in the dark for 30 minutes. For blank, the identical process was carried out, but ethanol was used in place of the juice. Using a UV-Visible spectrophotometer (UV-1600PC, Shanghai, China), the reduction in absorbance was detected at 517 nm. The DPPH radical scavenging activity was determined by using Equation (3):

$$\text{DPPH free radical scavenging activity}(\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

where, $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the juice.

## 3. Statistical Analysis

Each experiment was carried out three times. At $p < 0.05$, a two-way analysis of variance (ANOVA) was used to find significant mean differences. The information was presented as mean ± standard deviation (SD). Least Significant Difference (LSD) test was utilized to examine post hoc differences in mean values. The statistical tool for agricultural research (STAR) application was used to calculate the statistics.

## 4. Result and Discussion

### 4.1. Physicochemical Properties of Differently Processed Juices

#### Effects of Treatments on pH, Total Soluble Solids (TSSs) and Titratable Acidity (TA)

The pH, total soluble solids, and titratable acidity of the juice are related to its sensory quality; hence, alterations in these three qualities may have a negative effect on the juice's sensory quality. **Figure 2** shows pH, TSS, and TA values for differently treated juices. The pH of ripe and overripe pineapple juices before and after processing did not differ significantly ($p < 0.05$) (**Figure 2(a)**). The pH values for treated ripe and overripe pineapple juice varied from 4.70 to 4.73 and 4.52 to 4.53 respectively. The pH of the treatment solution may change as a result of ultrasonication due to the generation of reactive oxygen species (ROS) in the aqueous medium, such as hydrogen peroxide, nitrate, and nitrite, as described by Supeno and Kruus [26]. The pH of grape juice treated with ultrasonic waves at 20 kHz and 1500 watts at temperatures ranging from 32˚C to 45˚C did not change much [27]. In a recent experiment, Bhat *et al.* [7] also reported that the pH of kasturi lime juice was not significantly affected by ultrasonic treatment ($p > 0.05$) in any of the treatments.

One important pineapple quality factor that affects the sensory qualities of pineapple juice is total soluble solids (TSSs) as illustrated in **Figure 2(b)**. Mainly

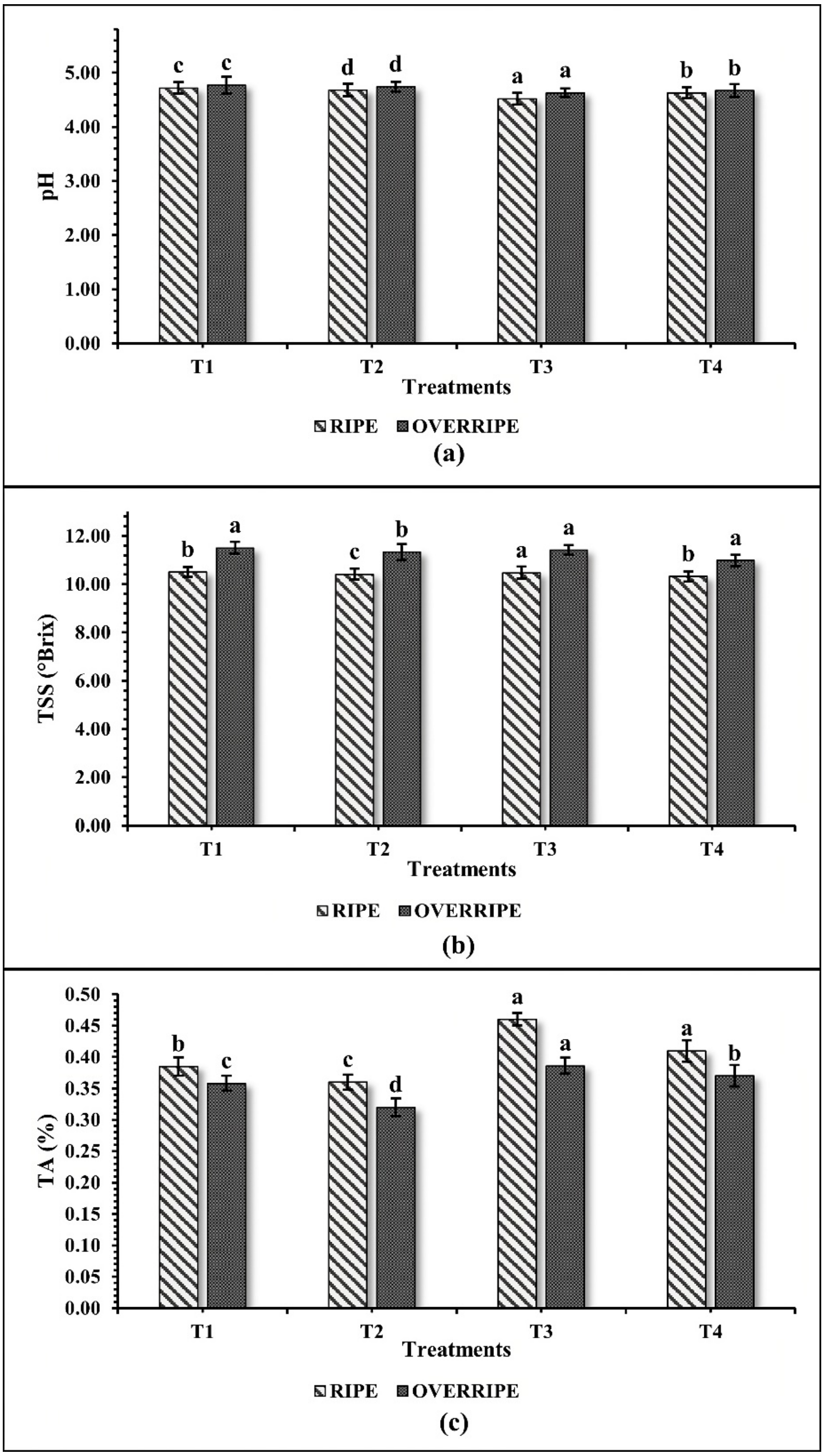


**Figure 2.** Effect of treatments on pH (a), TSS (b) and TA (c) of pineapple juice. Note: $T_1$ = Control (without treatment); $T_2$ = Pasteurization treatment (90˚C, 5 min); $T_3$ = Ultrasound treatment (37˚C, 5 min); $T_4$ = Pasteurization treatment (90˚C, 5 min) + Ultrasound treatment (37˚C, 5 min). Values with different letters are significantly different at $p < 0.05$ level.

depending on the fruit's maturity. Initially overripe fruit juice had a higher TSS (11.5˚Brix) than ripe fruit (9.70˚Brix), but it decreased after treatment. The combination of sonication and pasteurization led to a greater drop than sonication alone ($p < 0.05$), but the alterations were minor. This was due to the fact that ripe juice contained much fewer fermentable sugars than overripe juice, including d-glucose, d-fructose, and sucrose [28]. Saeeduddin *et al.* [16] also found no discernible differences in pear juice after conventional and ultrasound pasteurization at varied temperatures.

Bavisetty and Venkatachalam [20] also reported, the reduction of TSS in wax apple juice samples sonicated with subsequent pasteurization was greater than with sonication alone. All juices showed parallel titratable acidity (TA) changes when processed, independent of maturity (**Figure 2(c)**). In this study, there was no considerable ($p < 0.05$) increase in TA for pineapple juices after sonication and pasteurization treatments. Similar patterns were discovered in the juice of *Haematocarpus validus* [29]. These results agreed with the previous reports of Bavisetty and Venkatachalam [20].

## 4.2. Physical Appearances of Processed Juice

### Effects of Different Treatments on Color Values (L*, a*, and b*)

Fruit juice's color is a crucial sensory characteristic that can indicate nutritional quality or loss and affect consumer perception [21] [30]. Color is one aspect that consumers may use to determine the efficiency of fruit juice or pulp [31]. **Figure 3** displays the findings on the impact of the sonication and pasteurization processes on the color values (L*, a*, and b*) of pineapple juice. Results showed that when pineapple juice was treated with ultrasound, there was a substantial ($p < 0.05$) decrease in a* value (**Figure 3(a)**) and b* value (**Figure 3(b)**) but slightly increase in L* value (**Figure 3(c)**). Juice that was ripe at the start was lighter than juice that was overripe. Whereas pasteurization and sonication jointly decreased L*, with pasteurization having an especially significant impact on the lowering of L* for overripe juice, sonication alone had a less significant impact on L* of ripe or overripe juice across all examined sonication durations ($p < 0.05$). According to Aadil *et al.* [21], homogenizing by sonication raised L* due to inadequate precipitation of suspended particles, resulting in a high cloud value. A rise in temperature induced both enzymatic and nonenzymatic browning processes, which resulted in the decomposition of ascorbic acid and the caramelization of sugar, lowering L* [32]. Similar findings were reported by Bavisetty and Venkatachalam [20] who found that large reduction of L* value in ripe and overripe wax apple juice when processed by pasteurization and sonication. Cheng *et al.* [33] found the color shift in sonicated and carbonated guava juice, as well as significant changes in all color parameters, despite the fact that the naked eye was unable to identify these differences. Fonteles *et al.* [10] found that the breakdown of cell membranes during sonication results in the production of carotenoid-protein complexes, which produce a change in color.

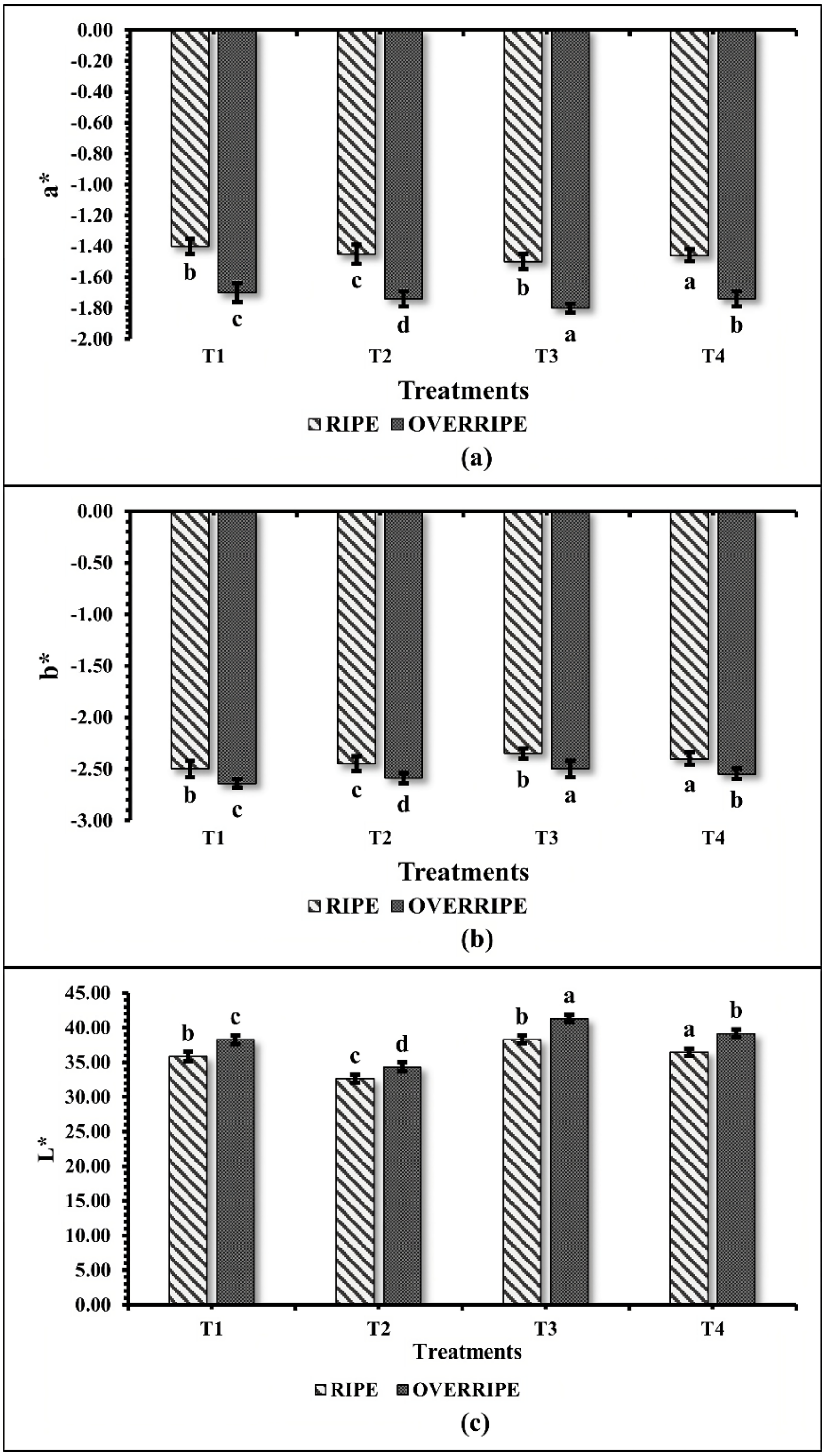


Figure 3. Effects of treatments on a* (a), b* (b) and L* (c) of pineapple juice. Note: $T_1$ = Control (without treatment); $T_2$ = Pasteurization treatment (90˚C, 5 min); $T_3$ = Ultrasound treatment (37˚C, 5 min); $T_4$ = Pasteurization treatment (90˚C, 5 min) + Ultrasound treatment (37˚C, 5 min). *L: value represents lightness from black (0) to white (100), a*: value represents color ranging from red (+) to green (−); b*: value represents yellow (+) to blue (−). Values with different letters are significantly different at $p < 0.05$ level.

### 4.3. Biochemical Alterations in Juices with Various Processing Methods

Bioactive substances for example flavonoids, anthocyanins, phenolics, ascorbic acid, and other antioxidants, add nutritional value to fruit juices. In overripe and ripe pineapple juices, antioxidant activity, ascorbic acid levels, and total phenolic content levels were all influenced by ultrasonication and pasteurization.

#### 4.3.1. Effects of Different Treatments on Ascorbic Acid (AA)

Ascorbic acid significantly aids in the prevention of numerous cardiovascular and cancer disorders [34]. Heat and oxygen are the primary causes of its deterioration. Figure 4 displays the results on the influence of various treatments on the ascorbic acid content of pineapple juices. Results showed that significant ($p < 0.05$) all sonicated juice samples contained more vitamin C than the control samples. Since sonication is a nonthermal procedure, certain degradation changes may be induced by cavitation effects; nonetheless, the loss of beneficial chemicals was shown to be negligible when compared to pasteurization. Ascorbic acid also breaks down at high temperatures, as can be seen in the reduction of ascorbic acid during pasteurization [20]. For instance, Zou and Jiang [35] reported that processed carrot juice using ultrasound (40 kHz, 0.5 W/cm$^2$) for 20 or 40 minutes. When the juice was processed for 20 and 40 minutes, the amount of ascorbic acid went up by 3.61 and 8.17 percent, respectively, compared to the fresh juice. Aadil *et al.* [21] reported that ascorbic acid concentration of sonicated apple juice treated for 60 and 90 minutes increased significantly ($p < 0.05$). According to research by Abid *et al.* [11], samples processed for 60 and 90 minutes had significantly higher ascorbic acid contents than samples processed for 30 minutes ($p < 0.05$). Previous studies on sonicated kasturi lime juice also showed

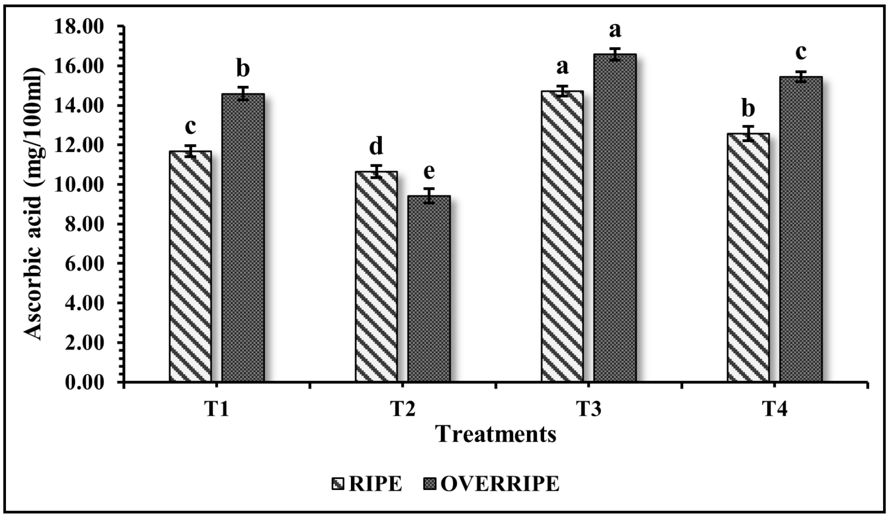


Figure 4. Effects of treatments on ascorbic acid of pineapple juice. Note: $T_1$ = Control (without treatment); $T_2$ = Pasteurization treatment (90˚C, 5 min); $T_3$ = Ultrasound treatment (37˚C, 5 min); $T_4$ = Pasteurization treatment (90˚C, 5 min) + Ultrasound treatment (37˚C, 5 min). Values with different letters are significantly different at $p < 0.05$ level.

an increase in vitamin C levels [7]. This rise in vitamin C might be caused by cavitation, which releases oxygen that has become trapped [33]. Our findings also agree with Bavisetty and Venkatachalam [20], who discovered that sonicated wax apple samples retained higher ascorbic acid content compared to control.

#### 4.3.2. Effect of Different Treatments on Total Phenolic Content (TPC)

Phenolic substances are very favorable to human health because they significantly lower the risk of several physiological and degenerative illnesses. **Figure 5** displays the findings on the impact of various treatments on the total phenolic compounds of pineapple juice. All sonicated juice samples displayed a significant increase in total phenols compared to the control sample. Previous studies on sonicated kasturi lime juice showed a substantial increase in TPC [7]. The breakdown of cell walls during ultrasound may be responsible for the rise in phenolic compounds, which makes it easier for intracellular components to be released. Moreover, this rise in phenolic compounds may be explained by the reaction between the aromatic ring of polyphenols and the hydroxyl radicals produced during sonification [36] [37].

#### 4.3.3. Effect of Different Treatments on DPPH Free Radical Scavenging Activity

Vitamin C and phenolic compounds play a major role in the DPPH radical-scavenging activity and antioxidant capacity of fruits and vegetables. These substances have the ability to scavenge dangerous free radicals and lower the risk of a number of diseases brought on by oxidative stress [21]. **Figure 6** displays the findings on the impact of various treatments on the DPPH free radical scavenging activity of ripe and overripe pineapple juices. All sonicated juice samples exhibit a substantial ($p < 0.05$) increase in DPPH free radical scavenging activity,

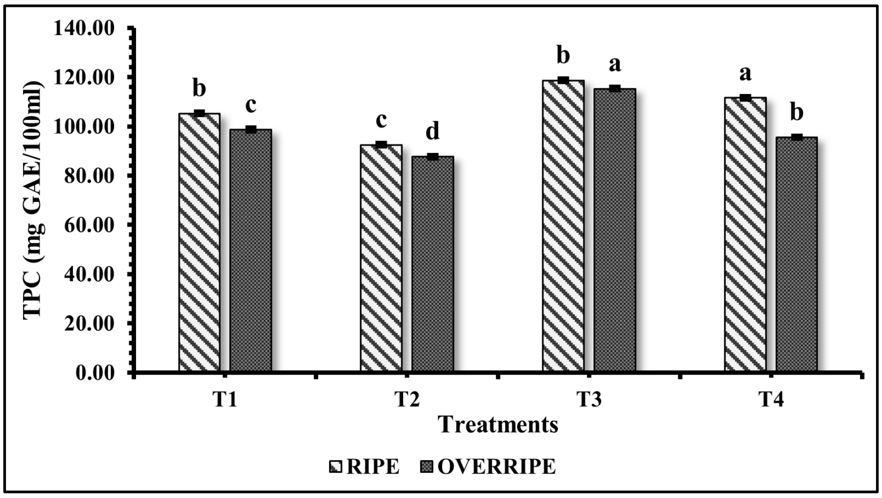


**Figure 5.** Effects of treatments on total phenolic content (TPC) of pineapple juice. Note: $T_1$ = Control (without treatment); $T_2$ = Pasteurization treatment (90˚C, 5 min); $T_3$ = Ultrasound treatment (37˚C, 5 min); $T_4$ = Pasteurization treatment (90˚C, 5 min) + Ultrasound treatment (37˚C, 5 min). Values with different letters are significantly different at $p < 0.05$ level.

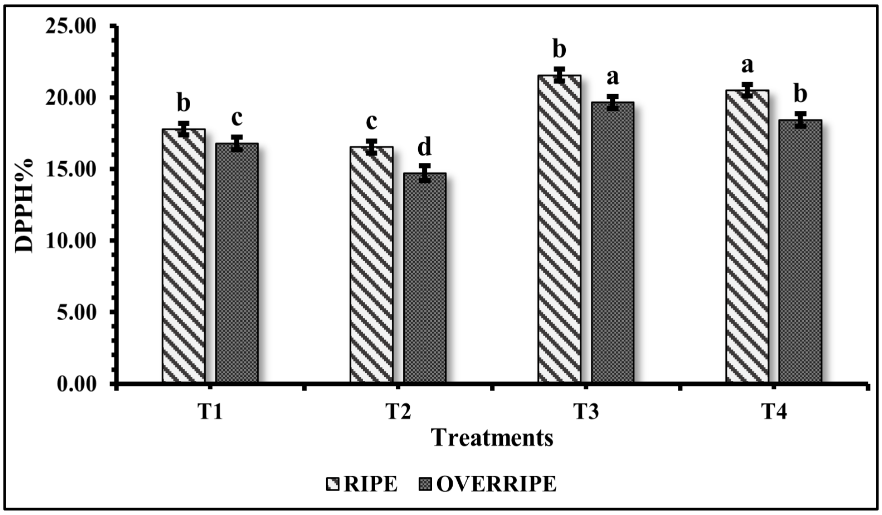


**Figure 6.** Effects of treatments on DPPH% of pineapple juice. Note: $T_1$ = Control (without treatment); $T_2$ = Pasteurization treatment (90˚C, 5 min); $T_3$ = Ultrasound treatment (37˚C, 5 min); $T_4$ = Pasteurization treatment (90˚C, 5 min) + Ultrasound treatment (37˚C, 5 min). Values with different letters are significantly different at $p < 0.05$ level.

which is similar to the findings for sonicated kasturi lime juice, as shown in **Figure 6** [7]. Bavisetty and Venkatachalam, [20] reported the same pattern of decreased antioxidant activity from wax apple juice by sonication and subsequent pasteurization. This increase may be attributable to the higher quantity of phenolic chemicals created by cavitation during sonication. There was a high correlation between antioxidant concentration and phytochemical content, particularly ascorbic acid and phenolic acid. According to Gil *et al.* [38] and Crozier *et al.* [39], the antioxidant potential of fruits is due to ascorbic acid, phenols, and flavonoids. In many plant species, previous studies have shown a link between total phenolic content and antioxidant activity [40] [41].

## 5. Conclusion

The physicochemical qualities of pineapple juices extracted at various maturities were enhanced by sonication alone or in conjunction with pasteurization. The benefits of sonication become more apparent with continued processing. In contrast, pasteurization significantly lowered the antioxidant activity of juices. Moreover, sonication is less expensive than industrial thermal processing, which is both time-consuming and energy-intensive. As a fruit juice processing procedure, sonication (alone or in combination with pasteurization) offers advantages over pasteurization.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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