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ORIGINAL PAPER

# Optimization of thermosonication processing of pineapple juice to improve the quality attributes during storage

**Thatchajaree Mala[1] · Muhammad Bilal Sadiq[2] · Anil Kumar Anal[1]**

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## Abstract

The microbial and functional quality attributes of fresh pineapple juice were studied under conventional and ultrasound pasteurizations. Fresh pineapple juice was subjected to thermosonication (TS) treatments with processing variables of temperature (25–65 °C), time (2–10 min) and amplitude (30–70%). Response variables: total phenolic content (TPC), bromelain activity and microbial inactivation were optimized using response surface methodology. The optimized TS treatment conditions for juice were 62.33 °C, 2 min and 35.32% amplitude, which corresponded to bromelain activity of 63.24 U/100 mL of juice, TPC of 177.3 µg GAE/100 mL, total plate count 2.74 log CFU/mL and yeast & mold count 2.301 log CFU/mL. Shelf-life of TS treated, pasteurized and control pineapple juices was evaluated for 28 days at 4 °C. TPC, ascorbic acid and protein contents of TS treated juice were significantly higher than pasteurized juice after 28 days of storage. Due to high efficiency in the retention of nutritional and quality parameters, TS processing can be opted by juice processing industries as an alternative to convention pasteurization.

**Keywords** Thermosonication; pasteurization · Pineapple juice · Shelf life · Preservation

## Introduction

The vegetable and fruit juices have recently gained considerable consumer's interest due to the presence of health promoting bioactive compounds and ease of consumption especially for packaged juices [1]. Pineapple (*Ananas cosmosus*) is one of the most important tropical fruits and its juice is famous, having sweet and sour taste as well as potential to various health benefits. Pineapple juice is a rich source of minerals and antioxidants such as ascorbic acid, carotenoids, and flavonoids, which reduce the incidence of cardiovascular and chronic degenerative diseases associated with oxidative damage [2]. Bromelain is the major proteolytic enzyme complex that is found predominantly in pineapple [3]. The health benefits of pineapple juice are mainly associated with its antioxidants and bromelain enzyme. The thermal processing or commercial pasteurization reduces the antioxidant potential of pineapple juice and degrade the bromelain [1]. Therefore, there is a dire need to implement alternate juice processing technologies which can retain the antioxidants, nutritional and sensory attributes [1, 4].

The conventional pasteurization (CP) method has long been used for the shelf life extension of a variety of foods and beverages [5]. Due to high processing temperature, CP reduces the quality and nutritional values of processed foods [6, 7]. To overcome the concerns associated with CP, various nonthermal technologies such as ultrasonication, high hydrostatic pressure, pulse electric field and high pressure homogenization have been practiced to retain the quality and nutritional profile of juices [1, 8].

Ultrasonication has recently been explored in food processing, being simple, reliable, environmental friendly and highly effective in achieving microbial decontamination and preservation [9, 10]. The ultrasonic energy propagates and generates cavitation in the solvent [11]. Among novel food processing techniques, ultrasound is combined with heat (temperatures ≥ 50 °C), and generally termed as thermosonication (TS) [12]. TS has been considered a potential method for the inactivation of microorganisms in fruit juices and drinks, due to synergistic effects of heat and the sound waves

✉ Anil Kumar Anal
anilkumar@ait.asia; anil.anal@gmail.com

[1] Department of Food, Agriculture and Bioresources, Food Engineering and Bioprocess Technology, Asian Institute of Technology, PO Box 4, Klong Luang 12120, Thailand

[2] School of Life Sciences, Forman Christian College (A Chartered University), Lahore 54600, Pakistan

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[13, 14]. To ensure the effectiveness of the TS treatment, it is necessary to consider variables such as the pH of the product, amplitude, temperature, and time of the treatment [15, 16]. Thermosonication has been reported to enhance the shelf life of fruit juices and dairy based drinks with minimal changes in nutritional and sensory properties [17].

Thermosonication processing of fruit and vegetable juices have been extensively explored, however the application of TS processing to retain the quality and nutritional attributes of pineapple juice during storage has not yet been reported. The aim of this study was to evaluate the effect of TS processing conditions and commercial pasteurization on nutritional and quality attributes of pineapple juice during the storage.

## Material and methods

### Pineapple juice preparation

Fresh pineapples (*Ananus comosus* L.) of Smooth Cayenne cultivars were purchased from the local market of Pathum Thani province, Thailand. The pineapples were peeled, sliced into chunks and juice was obtained by using laboratory scale juice extractor (MJ-SJ01 Panasonic, Malaysia). The juice was filtered through sterilized double layered muslin cloth and stored at 4 °C in sterilized airtight 100 mL bottles for maximum 24 h till further processing [7].

### Optimization of thermosonication

TS processing of pineapple juice was carried out in 150 mL conical flasks containing 80 mL of juice and subjected to an ultrasonic processor with 13 mm probe diameter (UP200S, 200 W, Hielscher, Teltow, Germany) at a fixed frequency of 24 kHz. TS processing of pineapple juice was carried out using three independent factors: TS temperature (25–65 °C), TS time (2–10 min), ultrasonic amplitude (30–70%) [7]. The temperature was maintained by using a digital temperature water bath (JSWB-22 T, JS Research Inc., Korea) and TS of juice was started when the set temperature was reached. All samples were immediately cooled down to 4 °C in ice water and stored in sterilized bottles, at 4 °C.

Box-Behnken experiment design (BBD) was implemented by using Design Expert 7.0 (Stat-Ease Inc., Mineapolis, USA) to optimize the TS processing of pineapple juice. The temperature ($X_1$, 25–65 °C), time ($X_2$, 2–10 min) and amplitude ($X_3$, 30–70%) were independent factors whereas, bromelain activity (Units/100 mL), total phenolics content (gallic acid equivalent µg/100 mL), total plate count (log CFU/mL) and yeast & mold count (log CFU/mL) were response variables (Table 1).

#### Bromelain activity

Ammonium sulfate precipitation was used for separation of enzyme/peptides from TS processed juice by following the method described by Gautam et al. [18] with some

**Table 1** Effects of thermosonication processing of pineapple juice on response variables determined by Box-Behnken design

| Run | Temperature (°C) | Time (min) | Amplitude (%) | Analytical responses | | | |
|---|---|---|---|---|---|---|---|
| | | | | Bromelain activity (units/100 mL) | Total phenolics (µg GAE/100 mL) | Total plate counts (log CFU/mL) | Yeast & mold counts (log CFU/mL) |
| 1 | 25.00 | 2.00 | 50.00 | 80.06 | 188.57 | 3.66 | 3.90 |
| 2 | 25.00 | 6.00 | 30.00 | 78.74 | 188.07 | 3.71 | 3.91 |
| 3 | 65.00 | 2.00 | 50.00 | 66.60 | 175.82 | 2.38 | 2.30 |
| 4 | 45.00 | 6.00 | 50.00 | 74.95 | 180.57 | 3.47 | 3.69 |
| 5 | 45.00 | 6.00 | 50.00 | 74.53 | 181.32 | 3.39 | 3.68 |
| 6 | 25.00 | 6.00 | 70.00 | 76.32 | 185.07 | 3.51 | 3.85 |
| 7 | 45.00 | 2.00 | 30.00 | 79.97 | 181.82 | 3.61 | 3.72 |
| 8 | 65.00 | 6.00 | 30.00 | 66.55 | 174.93 | 2.54 | 2.15 |
| 9 | 45.00 | 2.00 | 70.00 | 72.45 | 181.07 | 3.46 | 3.61 |
| 10 | 45.00 | 10.00 | 30.00 | 75.04 | 179.32 | 3.40 | 3.69 |
| 11 | 65.00 | 6.00 | 70.00 | 55.60 | 174.82 | 2.00 | 2.00 |
| 12 | 45.00 | 10.00 | 70.00 | 65.27 | 179.72 | 3.22 | 3.56 |
| 13 | 45.00 | 6.00 | 50.00 | 70.26 | 181.33 | 3.47 | 3.68 |
| 14 | 65.00 | 10.00 | 50.00 | 58.42 | 173.22 | 2.15 | 2.00 |
| 15 | 25.00 | 10.00 | 50.00 | 78.43 | 184.32 | 3.64 | 3.88 |

modifications. Ammonium sulfate was added 50% (w/v) to the processed juice maintained at 5–8 °C and incubated for 45 min with continuous stirring to allow precipitation. The samples were centrifuged (VARISPIN, NOVAPRO Co. Ltd., Korea) for 30 min at 4000×*g* and supernatant were discarded. Finally, the pellets were re-suspended in 0.01 M phosphate buffer (pH 7.0) and used to analyze the bromelain activity.

The proteolytic activity of the bromelain was evaluated by following the method of Murachi [19], using casein and L-tyrosine as a substrate and a standard, respectively with slight modifications. The enzyme solution (1 mL) was mixed with 5 mL (0.65%, w/v) of casein, in 0.05 M potassium phosphate buffer at pH 7.5. The reaction was carried out at 37 °C for 10 min and stopped by the addition of 5 mL of trichloroacetic acid (0.11 M). The mixture was then filtered through 0.45 µm filter (SC13P045S, HyundaiMicro, Korea) and obtained filtrate was mixed with 5 mL of 0.5 M sodium carbonate solution and 1 mL of 20% (v/v) Folin & Ciocalteu's Phenol reagent and further incubated in a water bath at 37 °C for 30 min. The mixture was filtered through 0.45 µm syringe filter and then absorbance was read at 660 nm by UV–VIS spectrophotometer (UNICAM, UV/VIS Spectrophotometer, UK). Tyrosine was used as a reference standard and bromelain activity was expressed as units/100 mL juice which were equated from µM of tyrosine equivalent liberated. One unit of bromelain activity is defined as 1 µg of L-tyrosine released in 1 min per mL of sample when casein is hydrolyzed under the standard conditions of 37 °C for 10 min.

#### Determination of total phenolics

Total phenolic content (TPC) of TS processed juice was measured by Folin–Ciocalteu colorimetric method as described by Saeeduddin et al. [7] with slight modifications. Folin-ciocalteu reagent, 1 mL (10%, v/v) was mixed with a 0.5 mL juice and left for 6 min. The sodium carbonate (2 mL, 5% w/v) was added to the mixture and incubated in dark for 60 min at room temperature (25 °C). The absorbance was read at 760 nm by UV–VIS spectrophotometer. Gallic acid was used as reference standard to prepare a calibration curve and results were expressed as µg of gallic acid equivalents (GAE) per 100 mL of juice.

#### Microbiological analysis

Food and Drug Administration's (FDA) standard method of Bacteriological Analytical Manual [20] was used to determine the microbial population of juice samples. Serial dilutions of juice were prepared and poured into sterile petri dishes with the help of a pipette. Total plate counts were determined by pour plate method using nutrient agar (NA) (Himedia Ltd., India). Molten agar (15 mL) was poured to each petri dish and mixed immediately followed by incubation for 48 h at 37 °C (JSGI-150 T, JS Research Inc., Korea). The bacterial colonies were counted and the results were expressed as log colony forming units (CFU) /mL of juice. Yeast & mold counts were determined by pour plate method using potato dextrose agar (PDA) (Himedia Ltd., India). All the media plates were placed in an incubator at 32 ± 1 °C. After 48 h of incubation, yeast & mold were counted in each plate and the results were expressed as log CFU/mL of juice.

### Shelf-life study of thermosonicated and pasteurized pineapple juice

Three pineapple juices (n = 3) were used to evaluate the shelf-life; control (fresh juice without any treatment), TS processed juice (treated with optimized TS condition) and thermally pasteurized juice (pasteurized at 95 °C for 1 min). The juices were aseptically filled into sterile glass bottles in a biosafety cabinet (6 V-T, Biosafety Cabinet-BIOHAZARD Class II MICROTECH, Labmicrotech, Thailand). The bottles were tightly capped, leaving minimum amount of headspace volume and stored at 4 ± 1 °C in a refrigerator. Juice samples were analyzed for pH, total soluble solids, sugar content, color attributes, TPC, antioxidant activity, ascorbic acid, protein content, bromelain activity and microbial count at 0, 7, 14, 21 and 28 days of storage.

#### Determination of total soluble solids and pH

Total soluble solid content of juice was determined by a hand refractometer (Master-M, Atago Co., Ltd., Japan) at 25 °C. The pH was analyzed by using a digital pH meter (3510 Bench pH/mV Meter, Jenway, UK). The juice sample (10 mL) was continuously stirred with a magnetic stirrer and pH was measured at 25 °C.

#### Total sugar content

Total sugar in the samples was determined according to the method of Nor et al. [21] with slight modifications. The diluted juice (1 mL) was mixed with 1 mL of 5% (w/v) phenol. Subsequently, 5 mL of a concentrated sulfuric acid (98%, v/v) was added, and the mixture was allowed to stand for 10 min. The mixture was then placed in a water bath for 20 min at 20 °C. Color developed was read at 480 nm using a UV–VIS spectrophotometer. D-glucose was used as reference standard to prepare a calibration curve.

#### Color determination

The color parameters were monitored during shelf-life study using a Hunter lab colorimeter (Hunter Lab

Spectro-colorimeter having illuminant D65, Model TC-PlllA, Japan) in the CIE L a b scale. The lab colorimeter was calibrated using a standard calibrated plate ($L^*$ 93.33, $a^*$ -0.91 and $b^*$ 1.46). The color coordinates monitored, were lightness values ($L^*$), redness values ($a^*$) and yellowness values ($b^*$) [22].

### Determination of total phenolic content and antioxidant activity

Total phenolic contents of juice was measured by Folin–Ciocalteu colorimetric method as described by Saeeduddin et al. [7] with slight modification. Free radical scavenging activity of the pineapple juice was measured using a method explained by Sadiq et al. [23] with some modifications. To a known aliquot (2 mL) of the juice, 2 mL of DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution (0.2 mM in methanol) was added, followed by incubation in dark for 30 min at room temperature (25 °C). The same procedure was conducted for blank but methanol was used instead of the juice. The decrease in the absorbance (due to the proton donating activity) was measured at 517 nm using the spectrophotometer. The DPPH radical scavenging activity was calculated as:

$$\text{DPPH radical scavenging activity}\,(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100 \quad (1)$$

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

### Determination of ascorbic acid

Ascorbic acid content was determined by iodine titration method [24] with slight modifications. Briefly, 2 mL of juice sample was mixed with 8 mL distilled water and 1 mL of starch indicator solution. The sample was then titrated with iodine solution (0.005 M). The endpoint of the titration was identified as the first permanent trace of a dark blue–black color due to the starch-iodine complex.

### Protein content and bromelain activity

The total protein content of juice was determined by Bradford [25] assay using bovine serum albumin (BSA) as a reference standard. The juice was centrifuged at 4000×*g* for 10 min and 100 µl of the supernatant was added to Bradford reagent and kept for 10 min at 25 °C. The absorbance was measured at 595 nm using UV–VIS spectrophotometer and protein concentration was determined by using BSA standard curve.

The proteolytic activity of the bromelain was determined by following the method of Murachi [19]. The assay was based on proteolytic hydrolysis of the casein by the enzyme. Bromelain hydrolyzed the casein to release L-tyrosine. One unit of enzyme activity was defined as the amount of enzyme, releasing a product equivalent to 1 µg of tyrosine/min/ml under the standard assay conditions and expressed as units/100 mL.

### Microbiological analysis

Food and Drug Administration's (FDA) standard method of Bacteriological Analytical Manual [20] was used to determine the microbial population of juice samples during each storage interval at 0, 7, 14, 21 and 28 days of storage.

## Statistical analysis

All experiments were performed in triplicates and the resulted were expressed as mean values with standard deviation. Statistical analysis was carried out by using SPSS statistical software package (IBM SPSS Statistics, 23.0, USA). One-way analysis of variance (ANOVA) and Tukey's HSD test were carried out to determine the significant differences ($p < 0.05$) among means observations.

# Results and discussions

## Optimization of thermosonication processing of pineapple juice

The effects of TS processing variables (temperature, time and amplitude) on responses variables (bromelain activity, TPC, total plate count and yeast & mold count) are presented in Table 1. Bromelain activity and TPC of TS processed pineapple juice ranged from 55.60 to 80.06 Units/100 mL and 173.22–188.57 µg GAE/100 mL of juice, respectively. The bacterial and fungal counts ranged from 2.0 to 3.71 and 2.0–3.91 log CFU/mL, respectively (Table 1). The effects of TS processing factors; temperature ($X_1$), time ($X_2$) and ultrasonic amplitude ($X_3$) on response variables were determined by using second order quadratic polynomial equations (Eqs. 2–5)

$$Y_{Bromelain\ activity} = 73.25 - 8.30X_1 - 2.74X_2 - 3.83X_3 - 3.13X_1^2 \quad (2)$$

$$Y_{Total\ phenolics} = 181.08 - 5.91X_1 - 1.34X_2 \quad (3)$$

$$Y_{Total\ plate\ counts} = 3.45 - 0.68X_1 - 0.087X_2 - 0.13X_3 - 0.48X_1^2 \quad (4)$$

$$Y_{Yeast\ \&\ mold\ counts} = 3.69 - 0.89X_1 - 0.049X_2 - 0.056X_3 - 0.070X_1X_2 - 0.67X_1^2 \quad (5)$$

The results of analysis of variance indicated that the applied quadratic models for bromelain activity, TPC and microbial loads were significant ($p < 0.05$) with correlation coefficient ($R^2$) values of 0.9803, 0.9918, 0.9936 and 0.9987, respectively (Table 2). Lack of fit was insignificant which indicated that applied models were well fitted to the experimental data.

All TS processing parameters (temperature, time and amplitude) significantly ($p < 0.05$) affected the response variables (bromelain activity, TPC, total plate and yeast & mold count). However, the TS amplitude did not significantly influence the TPC of pineapple juice. The interactive effects of TS processing parameters on response variables are presented as 3D response surface plots (Figure S1–S4). Bromelain activity, total phenolics and microbial count decreased with the increase in temperature, time and amplitude. The decrease in bromelain activity and TPC by TS processing of pineapple juice was attributed to an additive effect of cavitation and heat, which caused protein denaturation and reduction of specific enzyme activity [9, 25–27]. TS processing of pineapple juice resulted a decrease in microbial count. The results were in agreement with the findings of Kiang et al. [28], who reported a significant reduction in microbial load of mango juice at 60 °C ultrasound-pasteurization treatment. Increase in temperature and TS processing time can lead to significant reductions in microbial count [16, 28].

The optimized TS processing conditions were obtained by using desirability function of Design Expert. The optimized conditions for TS processing of pineapple juice were TS temperature 62 °C (temperature), 2 min (time) and 35% (amplitude of sonication). At this condition, bromelain activity, total phenolic content, bacterial and yeast & mold counts were predicted as 71.94 units/100 mL, 176.37 µg GAE/100 mL, 2.76 log CFU/mL and 2.55 log CFU/mL respectively. The experimental values obtained at optimal TS conditions for bromelain activity, TPC, bacterial and yeast & mold counts were 63.24 units/100 mL, 177.3 µg GAE/100 mL, 2.74 log CFU/mL and 2.301 log CFU/mL, respectively.

## Shelf-life study of thermosonicated and pasteurized pineapple juice

### pH, total soluble solid (TSS) and total sugar

Figure 1 illustrates the changes in the pH, total soluble solid (ºBrix) and total sugar content in pineapple juice during the 28 days of storage at 4 °C. The control sample (untreated juice) showed the significant decrease in pH level during 28 days of storage, which attributed to high microbial load in control. There was no significant difference ($p > 0.05$) in pH level between the TS treated and pasteurized juice samples from 0 to 7 days of storage. However, from 14 to 28 days of storage pH of TS processed pineapple juice was significantly lower ($p < 0.05$) than the thermally pasteurized juice. pH level of pineapple juice was reported to be directly associated with the microbial load and activity [29].

During the shelf-life study, from 7 to 28 days of storage TSS and total sugar content of TS processed and thermally

**Table 2** Analysis of variance (ANOVA) results for the effects of thermosonication parameters on bromelain activity, total phenolic content and microbial analysis of pineapple juice

| Source | Bromelain activity | | Total phenolics | | Total plate count | | Yeast & mold count | |
|---|---|---|---|---|---|---|---|---|
| | F value | p value | F value | p value | F value | p value | F value | p value |
| Model | 27.63 | 0.0010** | 66.80 | 0.0001*** | 85.59 | < 0.0001*** | 439.10 | < 0.0001*** |
| $X_1$ | 171.20 | < 0.0001*** | 561.56 | < 0.0001*** | 592.93 | < 0.0001*** | 3107.50 | < 0.0001*** |
| $X_2$ | 18.66 | 0.0076** | 28.81 | 0.0030** | 9.71 | 0.0264* | 9.50 | 0.0274* |
| $X_3$ | 36.51 | 0.0018** | 3.01 | 0.1432 | 22.97 | 0.0049** | 12.19 | 0.0175* |
| $X_1 X_2$ | 3.34 | 0.1271 | 1.37 | 0.2951 | 1.91 | 0.2259 | 9.58 | 0.0270* |
| $X_1 X_3$ | 5.66 | 0.0632 | 4.20 | 0.0956 | 4.46 | 0.0884 | 1.02 | 0.3590 |
| $X_2 X_3$ | 0.39 | 0.5583 | 0.67 | 0.4517 | 0.031 | 0.8668 | 0.049 | 0.8332 |
| $X_1^2$ | 11.22 | 0.0203* | 0.23 | 0.6527 | 137.29 | < 0.0001*** | 806.50 | < 0.0001*** |
| $X_2^2$ | 0.65 | 0.4554 | 1.28 | 0.3088 | 1.631 × 10–3 | 0.9694 | 0.018 | 0.8979 |
| $X_3^2$ | 0.77 | 0.4191 | 0.23 | 0.6527 | 0.24 | 0.6431 | 2.88 | 0.1502 |
| Lack of fit | 0.13 | 0.9340 | 3.69 | 0.2205 | 3.82 | 0.2144 | 165.82 | 0.0060 |
| | $R^2$0.9803 | | $R^2$0.9918 | | $R^2$0.9936 | | $R^2$0.9987 | |
| | $_{adj}R^2$0.9448 | | $_{adj}R^2$0.9769 | | $_{adj}R^2$0.9819 | | $_{adj}R^2$0.9965 | |

*Significant at 0.05 level

**Significant at 0.01 level

***Significant at 0.001 level. Temperature ($X_1$), time ($X_2$) and ultrasonic amplitude ($X_3$)

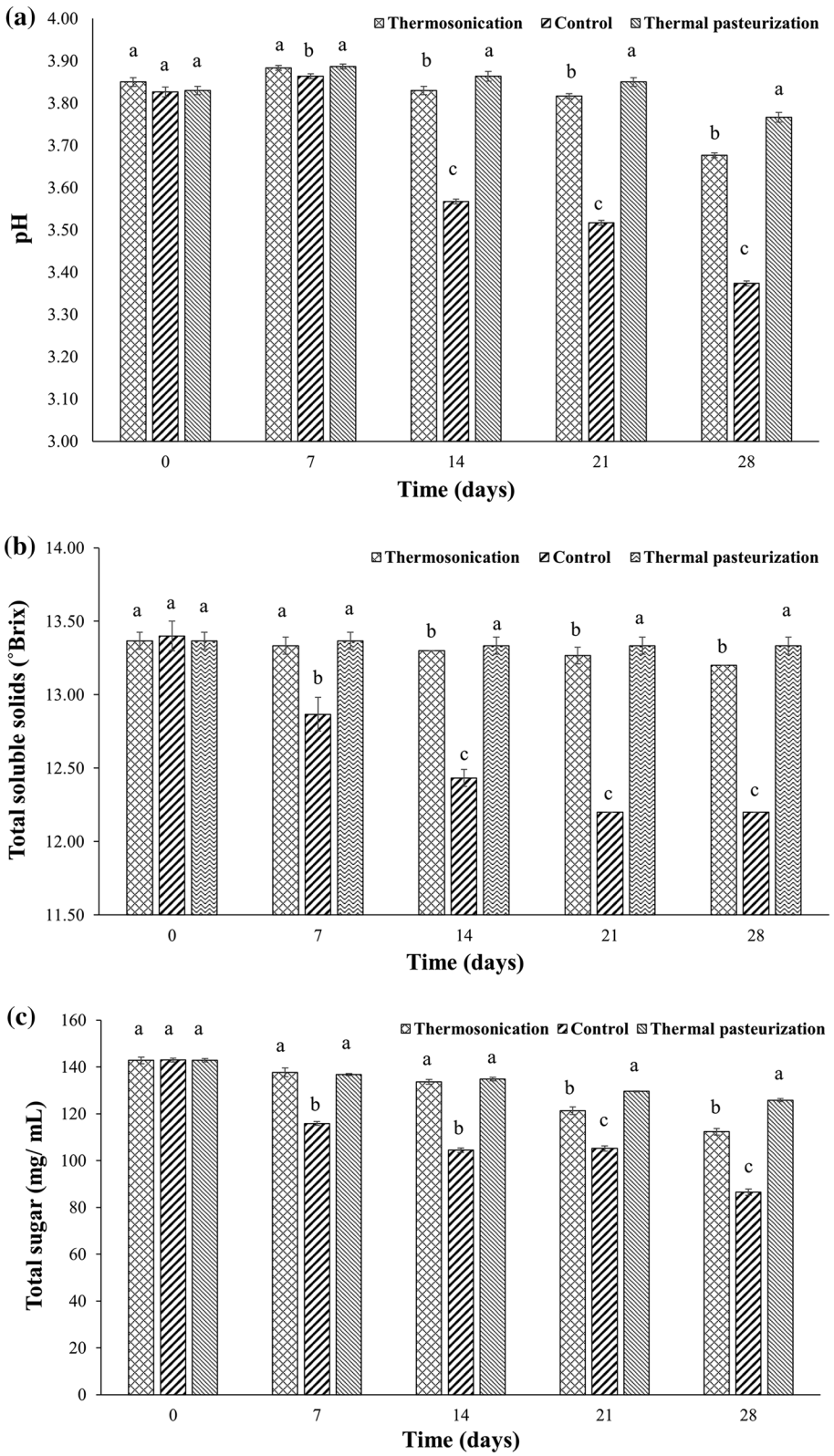


**Fig. 1** Changes in pH (**a**), total soluble solids (**b**) and total sugar content (**c**) in thermosonicated, untreated (control) and pasteurized pineapple juice stored at 4 °C for 28 days. Different letters above bars indicate significant differences ($p < 0.05$) among different treatments

pasteurized pineapple juice were significantly higher ($p < 0.05$) than the control sample. The pasteurized juice exhibited significantly higher ($p < 0.05$) total sugar content than TS treated juice after 21 days of storage. However, TSS of TS treated juice was significantly lower than the pasteurized juice sample from 14 to 28 days of storage. The

significantly lower TSS and total sugar content in control samples occurred due to spoilage and fermentation process, there was likely utilization of sugars, resulting in the production of acids, alcohol and carbon dioxide [30]. These results were in agreement with the previous reports [1, 29], where a decrease in TSS and sugar content of juice sample was reported during the storage.

### Color stability

Color changes (L*, a* and b* values) in thermosonicated, pasteurized and control pineapple juice during storage of 28 days are summarized in Table 3. There was a loss of lightness in all treatments. Untreated juice (control) showed significantly ($p < 0.05$) lower L* values than other treatments during storage and lightness was decreased with increase in storage interval. The significantly lower L* value of control juice was probably due to increase microbial and enzyme induced changes in carbohydrates. L* value of TS treated juice was significantly higher than conventional pasteurized juice for 0–7 days, however, after 14 days of storage the L* values of pasteurized juice were higher. Initially high L* value of TS processed juice was associated with low processing temperature, however from 14 to 28 days L* of TS processed juice was significantly lower than the pasteurized juice, which might be due to high microbial and enzymatic degradation of carbohydrates. The significant decrease in L* values of control juice was attributed to enzymatic browning [1]. Alcántara-Zavala et al. [31] reported that microorganisms and enzyme cause the degradation of carbohydrates and changes in food color. Rabie et al. [32], reported a decrease in L* value of pineapple juice after pasteurization due to nonenzymatic browning. The redness (a* value) was increased for TS treated and pasteurized juice after 28 days of storage, however all the samples had negative values for a* and b* which implied that they were in the green zone of hunter scale [29]. The yellowness (b* value) was decreased for TS treated and pasteurized juice after 28 days of storage which might be to ultrasonication treatment of pineapple juice [22].

### Total phenolic content and DPPH scavenging activity

There was a significant ($p < 0.05$) difference in TPC of control and treated pineapple juice during storage (Fig. 2a). TS treated juice was effective in retaining TPC as compared to the thermally pasteurized or the control juice sample at 4 °C during 28 days of storage. The results confirmed that the application of TS treatment preserved the TPC of juice during the storage. The TS treatment can retain the TPC better than the commercial pasteurization [1]. A lower TPC of pasteurized juice was associated with thermal degradation of polyphenolic compounds [33]. A similar trend was observed in DPPH radical scavenging activity of TS treated, pasteurized and control juice (Fig. 2b). TS treated pineapple juice exhibited high DPPH inhibition in comparison to control and pasteurized juice, which was associated with better retention of TPC of TS treated juice [34]. Yıkmış [35] reported that ultrasonicated watermelon juice retained high TPC and antioxidant activity than the control juice sample, which was due to the release of bonded phenolic compounds after ultrasonication treatment. High TPC and antioxidant activity of TS processed pineapple juice in comparison to control and thermal pasteurization were attributed to cavitation effect of sonication, which resulted in the mechanical breakdown of cell wall and release of free phenolic compounds [36].

**Table 3** Changes in color attributes (L * a* and b* values) of, thermosonicated, untreated (control) and pasteurized pineapple juice stored at 4 °C for 28 days

| Color attribute | Treatment | Time (days) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 7 | 14 | 21 | 28 |
| L* | TS | $18.11 \pm 0.05^{a}$ | $17.78 \pm 0.04^{a}$ | $17.39 \pm 0.09^{a}$ | $16.93 \pm 0.05^{b}$ | $16.01 \pm 0.07^{b}$ |
| | C | $17.76 \pm 0.11^{b}$ | $17.12 \pm 0.03^{c}$ | $16.64 \pm 0.3^{b}$ | $16.51 \pm 0.05^{c}$ | $15.09 \pm 0.05^{c}$ |
| | TP | $17.42 \pm 0.02^{c}$ | $17.39 \pm 0.10^{b}$ | $17.45 \pm 0.07^{a}$ | $17.39 \pm 0.02^{a}$ | $16.96 \pm 0.02^{a}$ |
| a* | TS | $-2.07 \pm 0.09^{b}$ | $-1.76 \pm 0.28^{a}$ | $-1.78 \pm 0.08^{a}$ | $-1.73 \pm 0.04$a | $-1.96 \pm 0.02^{ab}$ |
| | C | $-1.31 \pm 0.09^{a}$ | $-1.49 \pm 0.04^{a}$ | $-1.62 \pm 0.13^{a}$ | $-1.60 \pm 0.07^{a}$ | $-2.01 \pm 0.06^{b}$ |
| | TP | $-2.03 \pm 0.02^{b}$ | $-1.76 \pm 0.04^{a}$ | $-1.79 \pm 0.05^{a}$ | $-1.88 \pm 0.13^{b}$ | $-1.88 \pm 0.01^{a}$ |
| b* | TS | $-2.34 \pm 0.10^{b}$ | $-2.67 \pm 0.08^{b}$ | $-2.65 \pm 0.15^{b}$ | $-3.00 \pm 0.01^{b}$ | $-3.09 \pm 0.07^{b}$ |
| | C | $-2.69 \pm 0.08^{c}$ | $-2.79 \pm 0.08^{b}$ | $-2.82 \pm 0.11^{b}$ | $-2.92 \pm 0.07^{b}$ | $-3.00 \pm 0.30^{b}$ |
| | TP | $-2.03 \pm 0.01^{a}$ | $-2.11 \pm 0.02^{a}$ | $-2.31 \pm 0.01^{a}$ | $-2.50 \pm 0.01^{a}$ | $-2.46 \pm 0.02^{a}$ |

Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 3$). *TS* thermosonicated juice, *C* control, *TP* thermally pasteurized juice

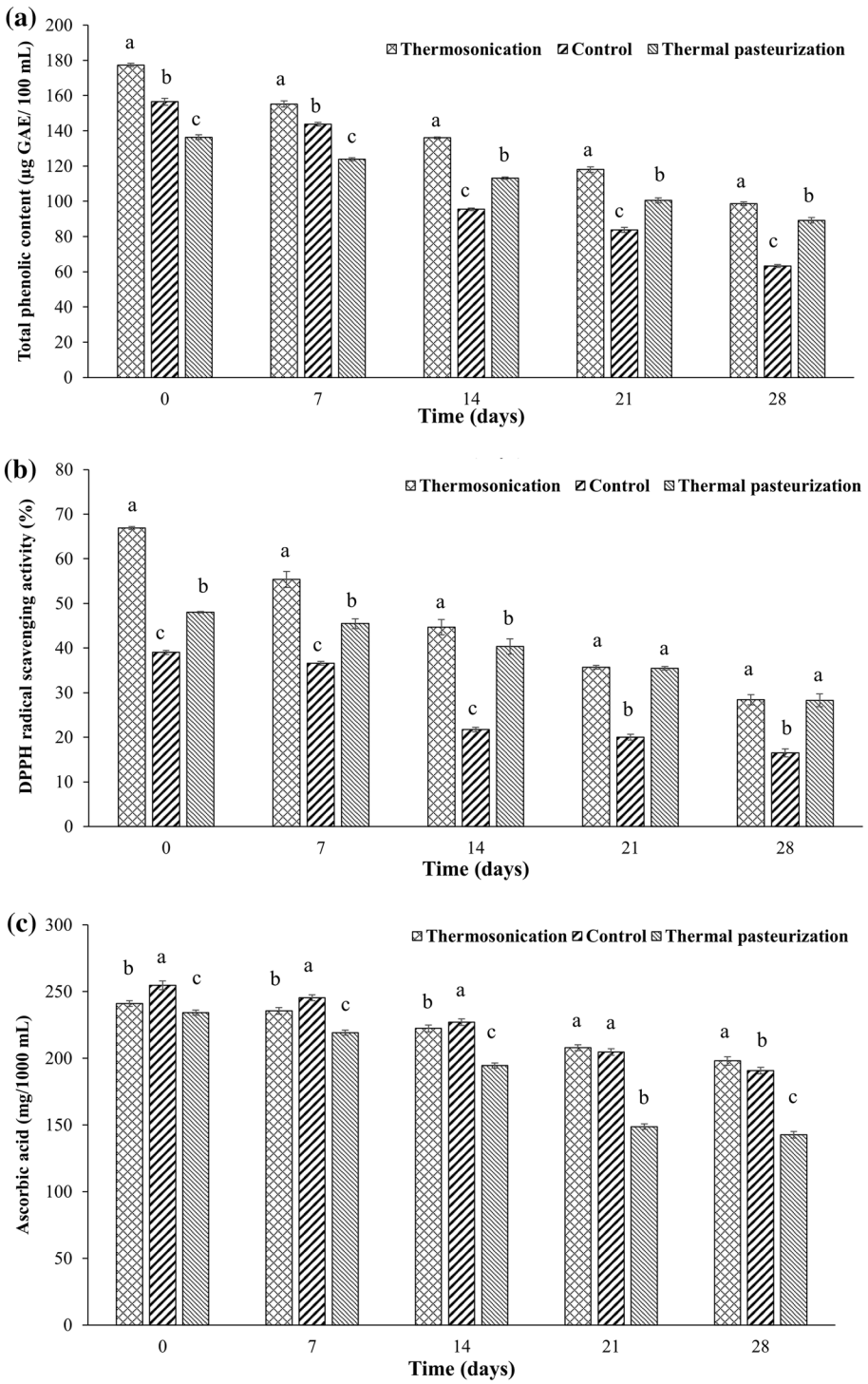


**Fig. 2** Changes in total polyphenol content (**a**), DPPH inhibition (%) (**b**) and ascorbic acid (**c**) in thermosonicated, untreated (control) and pasteurized pineapple juice stored at 4 °C for 28 days. Different letters above bars indicate significant differences ($p < 0.05$) among different treatments

## Ascorbic acid content

The ascorbic acid content decreased with storage time for all juice samples during the storage period (Fig. 2c). Pasteurized pineapple juice had a significantly lower ($p < 0.05$) concentration of ascorbic acid than TS treated and control juice due to the higher processing temperature [37]. The control juice sample initially showed the highest ascorbic acid content (0–14 days). However, at the end of storage the highest content of ascorbic acid was retained by TS treated juice. Wahia et al. [29] explained that TS treatment had a positive impact on ascorbic acid maintenance by the extraction of intracellular contents; however there was slight decrease until the end of the storage period. Aguilar

et al. [13] reported that ascorbic acid stability during the ultrasound processing is attributed to the degassing. Sonication results in a reduction of dissolved oxygen, a critical parameter influencing the stability of ascorbic acid. Tiwari et al. [37] evaluated ascorbic acid degradation during the sonication of orange juices and reported that ascorbic acid degradation was lower than 5% of the initial value, which was lower than thermally processed orange juice. Thermosonicated pineapple juice retained high ascorbic acid content compared to pasteurized juice sample, which was probably due to the removal of occluded oxygen from the pineapple juice by sonication treatment [38]. The decrease in ascorbic acid content of TS treated pineapple juice in comparison to control treatment, was attributed to thermal processing and acoustic effect [39].

### Protein content and bromelain activity

Protein content in pineapple juice was decreased with the increase in storage time and the protein content of control sample was significantly ($p < 0.05$) higher than TS treated and pasteurized juice (Fig. 3a). However, the protein content of TS treated pineapple juice was significantly ($p < 0.05$) higher than the pasteurized juice throughout the storage period (0–28 days). In comparison to control, the decrease in protein content of TS treated and pasteurized juice was attributed to degradation of protein due to thermal and ultrasonication processing, respectively [12]. The significantly ($p < 0.05$) lower bromelain activity was observed in thermally pasteurized juice (49.13 Units/100 mL) immediately after processing (0 day) than the other treatments, which was due to the thermal processing of juice. Costa et al. [12] reported a decrease in enzyme activity in pineapple juice after conventional heat pasteurization. Bromelain activity of TS treated juice was not significantly different ($p > 0.05$) when compared with the control sample at zero day (Fig. 3b), which indicated the stability of bromelain in TS treatment (62.33 °C) [40]. However, there was a gradual decrease in bromelain activity of all treatments during storage and after 28 days, bromelain activity of TS treated and

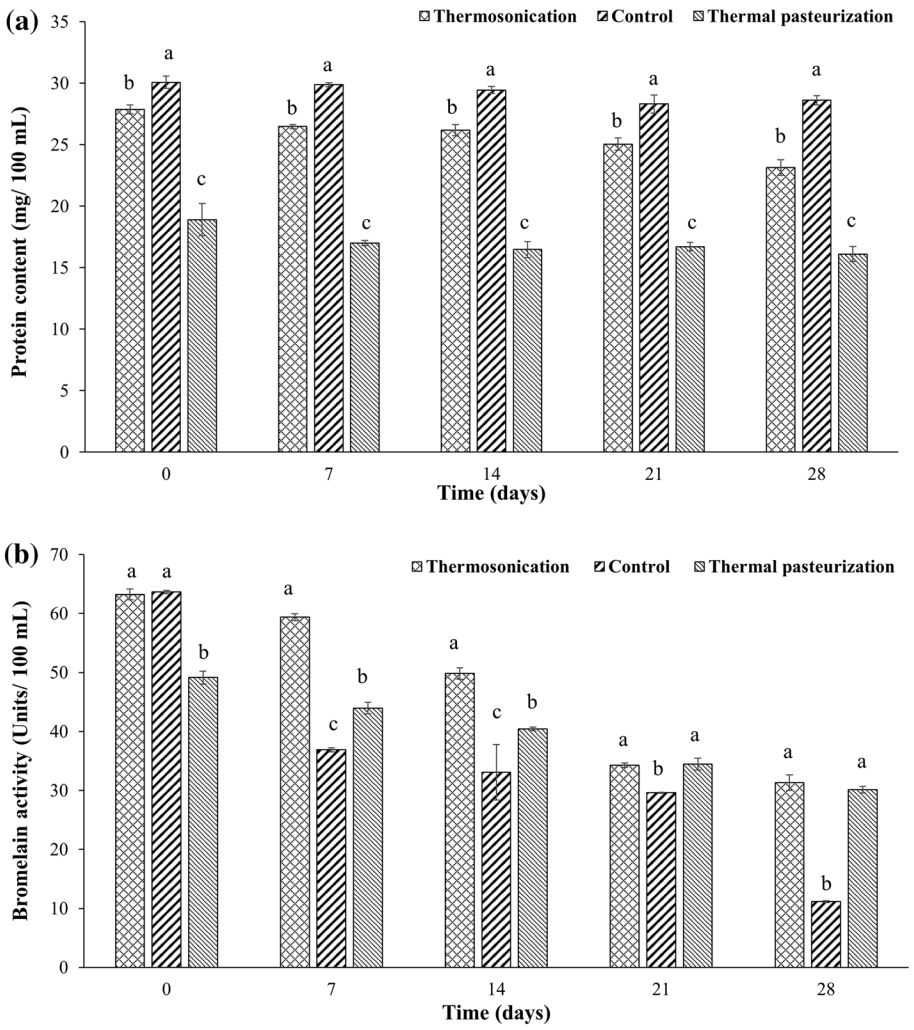


**Fig. 3** Changes in protein content (**a**) and bromelain activity (**b**) in thermosonicated, untreated (control) and pasteurized pineapple juice stored at 4 °C for 28 days. Different letters above bars indicate significant differences ($p < 0.05$) among different treatments

pasteurized juice was significantly higher than the control sample. Liao et al. [41] reported that enzymatic activity of pitaya juice decreased with increase in TS intensities. Moreover, more than 90% residual enzymatic activity was restricted in carrot, grapefruit and apple juices by TS treatment at 60 °C [25, 28, 36].

### Microbiological analysis of pineapple juice

The shelf-life and organoleptic quality of pineapple juice are influenced by the microbial growth. The total plate count and yeast & mold count in control (untreated) pineapple juice were significantly higher than the TS treated and pasteurized juice during storage of 28 days at 4 °C (Fig. 4). For TS treated, control and pasteurized juice, total plate counts at 0 day was 2.74, 4.71 and 1.93 log CFU/mL, respectively. Yeast & molds count, at 0 day was 2.30, 4.51 and 1.10 log CFU/mL for TS treated, control and pasteurized juice, respectively. Thermal pasteurization and TS treatment were found to be effective in delaying microbial growth of pineapple juice during storage. Pasteurization treatment was effective in lowering the microbial growth in pineapple juice during storage at 4 °C, however high processing temperature altered the nutritional and quality attributes of the juice [1].

The control sample revealed an initial total plate count and yeast & mold count of 4.71 and 4.51 log CFU/mL, respectively, which was increased to 6 log CFU/mL, during 1st week of storage. Increase in yeast count accelerates fermentation, causing a high production of ethanol and shortening the shelf life of the beverage [42].

TS treated juice sample restrict the microbial count throughout the storage period. The lower microbial count in TS treated juice might be due to ultrasound process in combination with heat (62.33 °C) that created the cavitation caused by the changes in pressure responsible for the destruction of bacteria. These observations are in agreement with previous reports, which reported that TS treatment can reduce the microbial levels in pineapple and orange juice [1, 43].

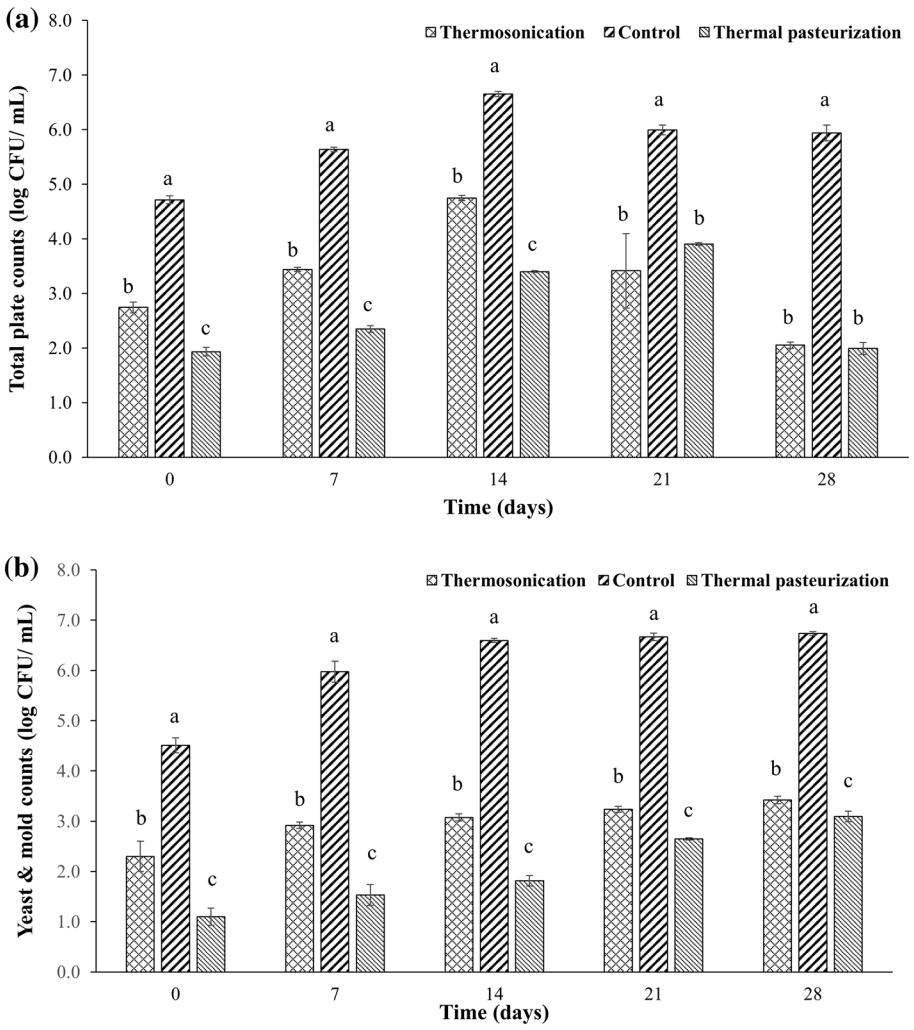


**Fig. 4** Total plate counts (**a**) and yeast & molds counts (**b**) in thermosonicated, untreated (control) and pasteurized pineapple juice stored at 4 °C for 28 days. Different letters above bars indicate significant differences ($p < 0.05$) among different treatments

## Conclusion

Commercial pasteurization of juices results in the loss of nutritional and quality attributes. The combination of ultrasonication and moderate temperature can serve as an alternative to conventional thermal pasteurization to preserve nutritional and quality attributes of juices. Due to low processing temperature, less treatment time and high efficiency in the retention of nutritional and quality attributes, TS processing can be opted by juice processing industries.

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**Authors contribution** TM performed all the experiments and wrote the manuscript under the supervision of AKA. MBS helped in designing the experimental plan, analysis of results and drafting the manuscript. AKA supervised the research and designed the experimental plan.

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**Data availability** Data transparency: all manuscript data is presented within manuscript and supplementary material.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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