

## Research Article

# Impact of Thermal, Ultrasonication, and Thermosonication Processes on the Quality Profile of Watermelon-Beetroot Juice Blend: A Comparative Study

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Fruit juices are popular beverages that provide various health benefits due to their rich nutritional profile, but they are prone to microbial spoilage and quality deterioration. Thermal pasteurization is the conventional method to preserve fruit juices, but it causes undesirable changes in the physicochemical and nutritional value of the juices. Therefore, there is a need to develop alternative methods to ensure the microbial safety and quality of fruit juices. The aim of this study was to investigate the impact of thermal (95-100°C for 4 min), ultrasonication (US) (25 kHz for 5 and 10 min), and thermosonication (TS) (25 kHz at 40 and 50°C) processes on the quality profile of watermelon-beetroot juice blend, a novel juice formulation with enhanced nutritional and functional properties with 50:50 formulation. The samples were analysed for physicochemical (colour, pH, total soluble solids, and titratable acidity), bioactive (phenolic, flavonoid, antioxidant, and ascorbic acid contents), and microbiological (total plate count and yeast/molds) properties. The results showed that all the processed samples retained high total phenolic (756.33-842.33 µg GAE/g), total flavonoid (435.33-512.67 µg CE/g), and ascorbic acid (45.23-50.34 mg/100 mL) contents along with a high antioxidant potential (total antioxidant capacity (274.14-305.33 µg AAE/g) and DPPH radical scavenging activity (33.05-42.18%)) while preserving the normal physicochemical characteristics and decreasing the microbial counts of all the processed blend juices. In conclusion, the US treatment (10 min) produced the juice blends with the best quality. The findings of this research suggest that thermal, US, and TS processes are promising technologies for the preservation of fruit juices and that watermelon-beetroot juice blend is a novel juice formulation with high nutritional and functional value. The results of this research might be useful to the processed fruit juice industry and the consumers who are looking for healthy and safe fruit juices.

### 1. Introduction

Perishable fruits and vegetables are a rich source of carbohydrates, dietary fibres, vitamins, minerals, bioactive compounds, and essential oils. The various components of these perishable commodities can be used to enhance the nutritional and functional characteristics of food and feed [1, 2]. Fruit juices are becoming increasingly popular due to their high nutritional benefits and rich sensory experience. They are good sources of essential vitamins, phenolic compounds, carotenoids, and anthocyanins, which are beneficial to different diseases [3–6]. However, they are prone to spoilage due to excessively high moisture content and the presence of high-value nutrients [7]. Beetroot is a highly nutritious food item that contains a large amount of bioactive compounds such as betalains, phenols, B vitamins, carotenoids, minerals, sugars, and inorganic nitrates [8, 9]. On the other hand, watermelon is also a fruit full of antioxidants and rich in nutrients. It is a good source of carotenoids mainly lycopene, which is the main reason of its red colour. It contains refreshing flavour and nutritious compounds, such as lycopene and antioxidants, which can lower the threat of cardiovascular diseases [10, 11]. It has become popular as a functional beverage for athletes as it contains amino acids especially citrulline and electrolytes which are ergogenic [12]. The pH of the juice is close to neutral and contains high moisture content which is favourable for microbial growth. Microbial growth reduces shelf life of the juice, and therefore, it is consumed in blend form with other juices with acidic pH [13].

Conventional methods such as pasteurization have been used in the food factories for decades to kill pathogens and reduce spoilage microbes in food products. While thermal treatments are mostly utilized to enhance the storage life of fruit and vegetable juices through microbial inactivation, their undesirable effects cannot be neglected [14]. Thermal treatments such as pasteurization (75 to 80°C) affect the sensory, physiochemical, and nutritional attributes and the consumer preference for fresh natural products that have led the technologists towards nonthermal technology applications [15]. Over the last decade, the use of emerging nonthermal technologies has increased such as pulsed electric field [16], US [17], pulsed light [18], and high-pressure processing. Among these emerging technologies, US is a widely applied technology that can preserve shelf life of the food products including fruit and vegetable juices without affecting their natural quality. High pressures, temperatures, and shear produced during cavitation process disrupt microbial cells, resulting in lowered microbial loads and improved food quality [19]. The US offers several benefits, which makes it an ideal candidate for the processing of fruit juices such as increasing the quality of fruit juices, lowering the energy consumption, lessening processing time, enhancing efficiency, and increasing the shelf life of the product. By combining US with heat, the efficacy can be improved resulting in better microbial inactivation. Studies have reported the increased efficacy of US when used in combination with heat, also called TS [20]. However, no study has reported the effect of TS and US on watermelon-beetroot juice blend. The blending of fruit juices is helpful not only to improve their flavour and taste but to also reduce the cost of the products, making them more affordable. While watermelon juice is rich in nutrients, there are high chances of microbial growth due to its high moisture content, and so, it must be blended with acidic fruit juices such as beetroot juice. Recently, blend juices have been developed using different fruit juices; however, none of the studies have reported a blend using watermelon and beetroot juices. Therefore, the present study was focused on formulating a watermelon-beetroot blend juice with an aim to cater the consumer's preference towards naturally produced fresh juices. The main objective of this study was to investigate and compare the impact of US, TS, and thermal treatments on bioactive compounds and physicochemical and microbial properties of watermelon-beetroot juice blend.

#### 2. Materials and Methods

2.1. Chemical and Reagents. Different chemicals and reagents such as 2,6-dichlorophenolindophenol, aluminium chloride, sulfuric acid, sodium phosphate, ammonium molybdate, sodium nitrite, peptone water, nutrient agar, potato dextrose agar, and tartaric acid were supplied by Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). While ascorbic acid was procured from ACCU Standard Inc. (New Heaven, USA), other chemicals such as 2, 2 diphenyl-2-picrylhydrazyl, catechin, gallic acid, ethanol, methanol, phenolphthalein, sodium hydroxide, the Folin–Ciocalteu reagent, and sodium carbonate were purchased from Appli-Chem GmbH (Darmstadt, Germany). The analytical grade chemicals and reagents were used for this research.

2.2. Procurement of Raw Material. Fresh watermelon and beetroot were taken from the market of Faisalabad, Pakistan. After sorting watermelon and beetroots (damaged fruits were removed), they were thoroughly washed with distilled water to eliminate the dirt and dust. The peeling and trimming of beetroot were done using stainless steel knives. Then, it was subjected to an electric juice extractor (Sonashi, Marseille, France). The watermelon was cut into pieces and added to the domestic blender (Model: SPJ-501, Marseille, France) for juice formation. The six layers of sterilized muslin cloth were used for filtration of fresh juices by sieving (1 mm mesh size) to eliminate debris and solid particles. The juices were mixed in a ratio of 50:50 and subjected to further treatments.

2.3. Blend Processing Treatments. The juice blend was taken in a 250 mL glass beaker, and a controlled temperature water bath was used for thermal treatment at the following conditions, i.e., 95-100°C for 4 min. For US treatment, an ultrasonic homogenizer (JY96-IIN, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China) was used to set the power at 70% and frequency at 25 kHz for 5 and 10 min. Similarly, watermelonbeetroot juice blend was subjected to TS at 40 and 50°C with the help of an ultrasonic probe at a frequency of 25 kHz, power of 420 W, and time of about 5 and 10 min for both temperatures. The untreated samples were taken as control, and all the blend samples were placed in sterilized bottles for further analysis. The treatment plan of this research is presented in Table 1.

#### 2.4. Analysis

2.4.1. *Physicochemical Analysis*. All the physicochemical parameters were evaluated for the treated and controlled watermelon-beetroot blend samples in triplicates.

(1) Brix, pH, Colour, and Acidity. The pH meter (Model: ETS-D6, IKA laboratories, Germany) determined the pH of control and experiment samples of watermelon beetroot blend. The standard AOAC (1990) method was used to calculate the titratable acidity (TA) (%) following the details

TABLE 1: Treatment plan.

Treatment	Treatment conditions
$T_0$	Control (untreated)
$T_1$	Thermal treatment (100°C, 4 min)
$T_2$	Ultrasonication (25 kHz, 5 min, 25°C)
$T_3$	Ultrasonication (25 kHz, 10 min, 25°C)
$T_4$	Thermosonication (25 kHz, 5 min, 40°C)
$T_5$	Thermosonication (25 kHz, 10 min, 50 $^\circ \rm C)$

described by Yildiz [21]. A refractometer (Model: PAL-1, Atago Co., Ltd., Japan) measured the total soluble solids (TSS) at room temperature following the modification given by Aadil et al. [17].

(2) Colour Measurement. The hunter colorimeter (Model: Labsan-XE, Hunter Associates Labs, Inc. USA) was used to determine colour values (L \*, a \*, and b \*) of each watermelon-beetroot juice sample. The numerical values of L \*, a \*, and b \* helped to calculate  $\Delta E$ , which is denoted as slightness, redness, yellowness, and colour difference [22].

$$\Delta E = \sqrt{\left[ (L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2 \right]}.$$
 (1)

2.4.2. Determination of Total Phenolic Content (TPC). TPC were measured using the method elaborated by Adulvitayakorn et al. [23] using the colorimetric Folin–Ciocalteu reagent. A spectrophotometer was used to determine TPC at 760 nm wavelength, and the results were expressed in terms of standard, i.e., microgram gallic acid equivalents per gram ( $\mu$ g GAE/g).

2.4.3. Estimation of Total Flavonoid Content (TFC). TFC in watermelon-beetroot juice blends were determined following a protocol described by Aadil et al. [17]. The spectrophotometer was used to measure the absorbance at 415 nm wavelength, and the results were expressed as microgram catechin equivalents per gram ( $\mu$ g CE/g).

2.4.4. Estimation of DPPH Free Radical Scavenging Activity. The DPPH free radical scavenging activity of watermelonbeetroot blend was measured following the method of Aadil et al. [17]. The absorbance was determined with a spectrophotometer (517 nm wavelength). The proton-donating activity was responsible for the decrease in absorbance. The DPPH radical scavenging activity was arrived at using the following equation:

DPPH radical scavenging activity (%) = 
$$100 \times \left(A_0 - \left(\frac{A_1}{A_0}\right)\right)$$
.  
(2)

Here,  $A_1$  is the absorbance of the watermelon-beetroot blend, and  $A_0$  is the absorbance of the control.

2.4.5. Estimation of Total Antioxidant Capacity (TAC). TAC was measured by the protocol mentioned by Aadil et al. [17]. The proton-donating activity of the mixture was determined using a spectrophotometer at 695 nm wavelength. The ascorbic acid (AA) was taken as a standard (100–400  $\mu$ g/mL), and the results were expressed in AA equivalents ( $\mu$ g AAE/g).

2.4.6. Estimation of AA. The AA value of the treated and untreated watermelon-beetroot blend was measured using the AOAC titrimetric method. The AA of the treated blend was determined using redox titration, which involves oxidation and reduction reactions. By titrating a standard vitamin C with a standard dye solution until a pink colour appeared, the colouring factor was determined. The AA of the juice blend was calculated using mg AA/100 mL [24].

This formula was applied to calculate AA:

$$Vitamin C\left(\frac{mg}{100} mL\right) = \frac{Volume of sample used \times titre \times dye factor \times 100}{Sample reading \times aliquot of extract taken}.$$
(3)

2.4.7. Microbiological Analysis. To evaluate the microbial quality of blend juice, total plate and yeast mold counts were determined. They were performed following the FDA's Bacteriological Analytical Manual's standard procedure (FDA, 2001). Microbiological analyses were estimated in standard log colony-forming units/millilitre of juice (log CFU/mL) [25].

2.4.8. Statistical Analysis. The data of every measured parameter was statistically analysed. The analysed results were expressed as mean values  $\pm$  SD (standard deviation). The data was subjected to statistical analysis using a completely randomized design (CRD) and one-way ANOVA (analysis of variance) at a significance level of p < 0.05. Statistix 9.0 software determined the remarkable differences between mean values by the Tukey HSD test.

#### 3. Result and Discussion

3.1. Effect of Treatments on Physicochemical Parameters. Table 2 shows the results regarding TSS, TA, colour, and pH of all treated watermelon-beetroot juice blend samples. The treatments showed a nonsignificant (p > 0.05) impact on the pH (3.98-4.00) and TA (0.54-0.55) values. In contrast, thermal, US, and TS exhibited a significant (p < .05) influence on TSS (13.60-17.65), L \* (25.46-28.32), a \* (4.05-8.07), and b \* (5.28-9.40) values. This remarkable increase in TSS and colour values might be due to the polysaccharide breakdown into monosaccharides and oligosaccharides, and the pigment degradation caused by ultrasonic waves (US) and temperature (thermal, TS). Overall, the best treatment in terms of TSS and colour was found to be US (10 min) treatment. Our findings are in agreement with the previously published study which also documented similar results during physicochemical analysis of apple-grape juice blend subjected to US and TS treatments at 25 kHz and 25°C [26].

3.2. Effect of Treatments on TPC and TFC. The impact of thermal, US, and TS on TPC and TFC of watermelonbeetroot juice blend has been summarized in Table 3. The

TABLE 2: Impact of different treatments on physicochemical analysis of watermelon-beetroot blend.

Treatments	pН	ТА	TSS	$L^*$	<i>a</i> *	<i>b</i> *
T <sub>0</sub>	$3.98\pm0.11^{\rm NS}$	$0.54\pm0.02^{\rm NS}$	$13.60 \pm 0.05^{d}$	$25.46 \pm 0.03^{d}$	$4.05\pm0.05^{d}$	$5.28 \pm 0.01^{a}$
$T_1$	$3.99\pm0.12^{\rm NS}$	$0.53\pm0.01^{\rm NS}$	$14.63 \pm 0.04^{e}$	$26.53 \pm 0.08^{\circ}$	$5.06\pm0.04^{\rm c}$	$6.37\pm0.07^{\rm f}$
$T_2$	$3.99\pm0.13^{\rm NS}$	$0.54\pm0.06^{\rm NS}$	$16.64 \pm 0.13^{a}$	$27.23\pm0.55^{b}$	$7.07\pm0.08^{\rm f}$	$8.39\pm0.03^{\rm b}$
$T_3$	$4.00\pm0.14^{\rm NS}$	$0.55\pm0.08^{\rm NS}$	$17.65 \pm 0.09^{\circ}$	$28.32\pm0.07^a$	$8.07\pm0.03^{\rm b}$	$9.40 \pm 0.06^{\rm e}$
$T_4$	$3.98\pm0.13^{\rm NS}$	$0.54\pm0.03^{\rm NS}$	$16.55\pm0.11^{\rm b}$	$27.16\pm0.04^{b}$	$7.00\pm0.07^{\rm e}$	$7.36\pm0.05^d$
$T_5$	$3.99\pm0.12^{\rm NS}$	$0.54\pm0.09^{\rm NS}$	$16.23\pm0.11^{b}$	$27.12\pm0.03^{\rm b}$	$6.99\pm0.02^{a}$	$7.30\pm0.04^{\rm c}$

Note: The different letters (a–f) in the similar column indicated the noteworthy difference in treatment results. Abbreviations: NS: nonsignificant;  $T_0$ : control juice;  $T_1$ : thermal treatment (100°C, 4 min);  $T_2$ : US (25 kHz, 5 min, and 25°C);  $T_3$ : US (25 kHz, 10 min, and 25°C);  $T_4$ : TS (25 kHz, 5 min, and 40°C);  $T_5$ : TS (25 kHz, 10 min, and 50°C).

comparison of thermal ( $T_1$ : 100°C, 4 min), US ( $T_2$ : 25 kHz, 5 min,  $T_3$ : 25 kHz, 10 min), and TS ( $T_4$ : 5 min, 40°C,  $T_5$ : 10 min 50°C) showed significant results as compared to control  $(T_0)$ . The maximum surge in TPC was noticed in  $T_2$ (815.67  $\mu$ g GAE/g) and T<sub>3</sub> (842.33  $\mu$ g GAE/g) treatments. This increase might be attributed to ultrasonic treatment because this technique increased bound antioxidants such as phenolics which lead to increase TPC. The TS showed less increase in  $T_4$  (811.67  $\mu$ g GAE/g) and  $T_5$  (807.33  $\mu$ g GAE/g) treatments as compared to US while the least was demonstrated in  $T_1$  (675.67 µg GAE/g) treatment. The US treatment showed the highest increase in TFC due to the generation of shock and shear waves during cavitation process which has been reported to release free phenolic acids and amino acids and add hydroxyl radicals to aromatic structures [27]. Similarly, the maximum elevation was exhibited in TFC after US ( $T_2$ : 501.33  $\mu$ g CE/g and  $T_3$ : 512.67  $\mu$ g CE/g), followed by TS ( $T_4$ : 499.00  $\mu$ g CE/g,  $T_5$ : 495.67  $\mu$ g CE/g), and the lowest was found after thermal treatment ( $T_1$ : 399.33  $\mu$ g CE/g). The release of phenolic compounds due to the rupture of cell membranes of plant cells which occurs primarily due to the cavitation process might have increased the TFC. During US processing, the shear force is generated, and sharp changes in pressure and temperature are induced, causing cell wall disruption and release of polyphenols. Another reason behind the enhancement of TFC might be through production of hydroxyl radicals during bubble implosion [17]. Our findings agreed with the results of Bhat and Goh [28] who found an increase in the TFC content of grapefruit juice after these treatments.

3.3. Effect of Treatments on AA Content. The mean values in Table 3 reveal the impact of thermal, US, and TS on the AA content of watermelon-beetroot juice blend. The maximum increase was recorded in  $T_2$  (47.89 mg/100 mL) and  $T_3$  (50.34 mg/100 mL) samples after US (5 min, 10 min) treatment. The removal of trapped oxygen during the cavitation process of US could account for this increase in AA. The TS treatment showed less increase in  $T_4$  (46.71 mg/100 mL) and  $T_5$  (45.10 mg/100 mL) samples than US, and the lowest value was recorded in  $T_1$  (43.78 mg/100 mL). AA is a heatsensitive compound. The least values of AA in the case of thermal and TS treatments could be due to a rise in temperature during reactions and the production of certain com-

pounds that catalyse the AA's degradation pathways when oxygen is present. The degradation of AA caused by electrochemical reactions might have been aided by a high temperature. Our findings revealed a significant increase in AA values in processed samples which is compatible with Feng et al. [22] who also recorded similar results in processed peach juice at 40 kHz. Moreover, the US remarkably increased the AA content (4.70%) in guava juice and retained (80.39%) in bayberry juice at 20 kHz for 2-10 min [29, 30].

3.4. Effect of Treatments on Antioxidant Assays. Table 3 manifests the results regarding the impact of thermal, US, and TS on DPPH free radical scavenging activity and TAC. The thermal treatment ( $T_1$ : 29.47%) reduced the DDPH free radical scavenging activity as compared to control  $(T_0)$ : 33.05%). Similarly, TS treatment showed a negative impact, and DDPH values were reduced with increasing time and temperature in comparison to US-treated samples. The highest scavenging activity was observed in  $T_3$  (42.18%) and was followed by  $T_2$  (39.47%),  $T_4$  (39.21%),  $T_5$ (39.18%), and  $T_0$  (33.05%). The lowest value was found for  $T_1$  (29.47%) among all the samples. Our findings with respect to DDPH values were similar with the results of Wahia et al. [31] who found the highest DPPH free radical scavenging activity for US-treated strawberry juice samples. Moreover, the data analysis revealed a clear relationship between TPC and DDPH scavenging activity. The DPPH free radical scavenging activity of fruit juices depends on their phenolic compounds which have the ability to scavenge free radicals which are harmful for our body and thereby can lower the rate of oxidative stress-related diseases. The increase in AA and polyphenols during US processing might have increased the DPPH free radical scavenging activity.

Further, all the treatments showed a significant (p < 0.05) effect on TAC. The US-treated samples showed the highest TAC in comparison to TS and thermal treatments. Notable differences were found in TAC in treated samples compared to the control, viz.,  $T_3$  (305.33  $\mu$ g AAE/g),  $T_2$  (303.32  $\mu$ g AAE/g),  $T_4$  (298.67  $\mu$ g AAE/g),  $T_5$  (295.00  $\mu$ g AAE/g),  $T_0$  (274.14  $\mu$ g AAE/g), and  $T_1$  (255.55  $\mu$ g AAE/g). This increase in the values of TAC could be attributed to the cavitation process, which causes the greater release of matrix-bound phenolic compounds and AA content that could increase

Experiments	TPC (µg GAE/g)	TFC ( $\mu$ g CE/g)	AA content (mg/100 mL)	DPPH (%)	TAC (µg AAE/g)
$T_0$	$756.33 \pm 0.05^{b}$	$435.33 \pm 0.52^{\rm b}$	$45.23 \pm 0.09^{\circ}$	$33.05 \pm 0.93^{b}$	$274.14 \pm 1.03^{b}$
$T_1$	$675.67 \pm 0.06^{a}$	$399.33 \pm 0.54^{a}$	$43.78\pm0.05^a$	$29.47\pm0.88^a$	$255.55 \pm 1.55^{a}$
$T_2$	$815.67 \pm 0.05^{e}$	$501.33 \pm 0.53^{e}$	$47.89 \pm 0.02^{e}$	$39.47\pm0.13^{\rm e}$	$303.32 \pm 3.06^{e}$
$T_3$	$842.33\pm0.07^{\rm f}$	$512.67\pm0.60^{\rm f}$	$50.34\pm0.07^{\rm f}$	$42.18\pm0.36^{\rm f}$	$305.33\pm2.01^{\rm f}$
$T_4$	$811.67\pm0.04^d$	$499.00\pm0.53^d$	$46.71\pm0.08^d$	$39.21\pm0.41^d$	$298.67 \pm 1.53^{d}$
$T_5$	$807.33 \pm 0.04^{\circ}$	$495.67 \pm 0.62^{\circ}$	$45.10\pm0.03^b$	$39.18 \pm 0.28^{\circ}$	$295.00 \pm 1.00^{\circ}$

TABLE 3: Impact of different experiments on TPC, TFC, AA, DPPH, and TAC of watermelon-beetroot juice blend.

Note: The different letters (a-f) in the similar column indicated the notable difference in treatment results. Abbreviations:  $T_0$ : control juice;  $T_1$ : thermal treatment (100°C, 4 min);  $T_2$ : US (25 kHz, 5 min, and 25°C);  $T_3$ : US (25 kHz, 10 min, and 25°C);  $T_4$ : TS (25 kHz, 5 min, and 40°C);  $T_5$ : TS (25 kHz, 10 min, and 50°C).



FIGURE 1: Impact of different treatments on total plate count of watermelon-beetroot juice blend ( $T_0$ : control juice;  $T_1$ : thermal treatment (100°C, 4 min);  $T_2$ : US (25 kHz, 5 min, and 25°C);  $T_3$ : US (25 kHz, 10 min, and 25°C);  $T_4$ : TS (25 kHz, 5 min, and 40°C);  $T_5$ : TS (25 kHz, 10 min, and 50°C)).

antioxidant activity. In comparison to the US, the TS showed less increase in TAC possibly due to the high temperature and long exposure time. Our results agree with earlier findings as reported by Basumatary et al. [32] who found a similar increase in TAC values for US-processed grapefruit juice and attributed it to the inactivation of oxidative enzymes and release of phenolic compounds from the cell structure. Application of US and TS has been found to increase TAC values in different juices [33, 34].

3.5. Effect of Treatments on Microbiological Analysis. The impact of thermal, US, and TS treatments on the microbial quality of watermelon-beetroot blend is presented in Figures 1 (total plate count) and 2 (yeast and mold count). All the treatments showed a notable decrease in the total plate count of the samples (Figure 1). The maximum decline was documented in the total plate count of  $T_3$  (2.74 log CFU/mL) samples followed by  $T_2$  (3.02 log CFU/mL),  $T_4$  (3.07 log CFU/mL),  $T_5$  (3.10 log CFU/mL), and  $T_1$  (3.11 log CFU/mL). The US treatment was found to be the most effective in reducing total plate counts possibly due to the cavitation effect. The cavitation process is associated with the production of free radicals and a sharp increase in temperatures and pressures in localized micro regions, which

could lead to microbial deaths. These findings indicate a positive impact of US treatment on the microbial quality of watermelon-beetroot juice blend and agree with the findings observed by Kalsi et al. [30]. A decrease in the microbial counts observed in thermosonicated samples might be attributed to the temperature. These results are consistent with the findings of Umair et al. [35] who also found a similar reduction in total plate counts of the thermosonicated carrot juice samples and attributed the reduction to high temperature and process-induced impact on microbe cell membranes.

Further, all the treatments manifested a notable (p < 0.05) reduction in yeast and mold counts as presented in Figure 2. The maximum decrease in yeast and mold counts was noted in  $T_3$  (2.07 log CFU/mL), followed by  $T_2$  (2.20 log CFU/mL),  $T_4$  (2.23 log CFU/mL),  $T_5$  (2.27 log CFU/mL), and  $T_1$  (2.58 log CFU/mL). The lowest reduction was observed after thermal treatment  $T_1$ . The maximum reduction (p < 0.05) in yeast and mold counts was achieved after US with increasing exposure time possibly due to the cavitation process. These findings are similar to the results reported by Nadeem et al. [36] who also found a positive impact of US treatment on yeast and mold counts of carrot-grape juice blend. In comparison to our study,



FIGURE 2: Impact of various treatments on yeast and mold counts of watermelon-beetroot juice blend ( $T_0$ : control juice;  $T_1$ : thermal treatment (100°C, 4 min);  $T_2$ : US (25 kHz, 5 min, and 25°C);  $T_3$ : US (25 kHz, 10 min, and 25°C);  $T_4$ : TS (25 kHz, 5 min, and 40°C);  $T_5$ : TS (25 kHz, 10 min, and 50°C)).

Nadeem et al. [36] used a longer US exposure time (60-90 min), and increasing the processing time showed a clear decrease in the microbial activity of juice blend. Due to the cavitation process, free radicals are produced which have the capability to kill the microorganisms. Compared to the control samples, the application of TS also caused a remarkable (p < 0.05) reduction in the activity of yeast and mold counts. This reduction achieved in yeast and mold counts is consistent with the findings of C. L. Nguyen and H. V. H. Nguyen [37] who recorded a similar reduction and attributed it to high temperature and process-induced electroporation of microbial cell membranes. While US alone may have a low lethal effect on microorganisms, combining it with heat is more effective.

#### 4. Conclusions

The study reports the development of a novel watermelonbeetroot juice blend for better nutritional and microbial quality. It evaluated the impact of three treatments, viz., thermal, US, and TS on physicochemical, microbiological, and bioactive properties of the blend juice. A significant increase was recorded in antioxidant potential (TPC, TFC, TAC, and DDPH radical scavenging activity) and AA contents after US (25 kHz for 5 and 10 min) as compared to TS (25 kHz for 5 min at 40°C and 10 min at 50°C), thermal (100°C for 4 min), and control samples. All the samples retained normal values for pH, TSS, and TA. Among the treatments, US could be preferred due to its positive impact on the nutritive value of the blend whereas TS has some drawbacks in terms of reduced bioactive properties. A limited number of studies have evaluated the impact of US and TS treatments on the quality characteristics of blend juices. This is the first report on the development of a processed watermelon-beetroot juice blend and may be helpful in the production of innovative and natural beverages for increased microbial stability and valorisation of less desirable fruits and vegetables. Our results indicate that US processing can be applied in fruit juice processing industries for production of minimally treated blends to fulfil the demands of consumers. Further studies should focus on other research aspects and parameters of blend juices such as rheological studies.

#### **Data Availability**

All the derived data supporting the findings of this study are used in this manuscript.

#### **Conflicts of Interest**

The authors declare that they have no conflict of interest.

#### **Authors' Contributions**

Kinza Mukhtar was responsible for investigation, data generation, formal analysis, and writing of the draft manuscript. Brera Ghulam Nabi was responsible for investigation, data generation, formal analysis, and writing of the draft manuscript. Muhammad Faisal Manzoor was responsible for investigation, reviewing, writing, and editing. Sania Zia was responsible for interpretation, reviewing, writing, and editing. Zuhaib F. Bhat was responsible for interpretation, reviewing, writing, and editing. Shahzad Hussain was responsible for interpretation, reviewing, writing, and editing. Rana Muhammad Aadil was responsible for project facilitation, resources, data curation, supervision, reviewing, writing, and editing.

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