Ultrasound-assisted thermal processing: Microbial safety and physicochemical attributes of Tejuino, a Mexican heritage fermented beverage

Procesamiento térmico-asistido por ultrasonido: Seguridad microbiana y propiedades fisicoquímicas del Tejuino, bebida fermentada tradicional mexicana

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Highlights

- Tejuino is a traditional fermented beverage from Mexico with potential probiotic properties.
- Ultrasound-assisted thermal processing is an effective alternative to reduce microbial spoilage population in tejuino beverage.
- Ultrasound-assisted thermal processing promotes slight changes in physicochemical properties and antioxidants, in a temperature-dependent response.

Innovaciencia
ISSN: 2346-075X
E- ISSN: 2346-075X
Innovaciencia 2026; 1(14):e5567
http://dx.doi.org/10.15649/2346075X.5567

ORIGINAL RESEARCH

How to cite this article:
Anaya-Esparza LM, Solano-Cornejo MA, Villagrán Z, Ruvalcaba-Gómez JM, Silva-Jara JM, Martínez-Esquivias F, Aurora-Vigo EF. Ultrasound-assisted thermal processing: Microbial safety and physicochemical attributes of Tejuino, a Mexican heritage fermented beverage. Innovaciencia 2026; 14 (1): e5567
http://dx.doi.org/10.15649/2346075X.5567

Received: 8 August 2025
Accepted: 16 December 2025
Published: 3 March 2026

Keywords:

Traditional beverage; maize beverage; nixtamalization process; commercial tejuino; ultrasound treatment; low thermal treatment; microbial safety.

Palabras clave:

Bebida tradicional; Bebida de maíz; nixtamalización; tejuino comercial; tratamiento de ultrasonido; tratamiento térmico a bajas temperaturas; Seguridad microbiana.

ABSTRACT

Introduction. Tejuino is an artisanal Mexican fermented corn beverage with limited shelf life, challenging industrial production. Research on microbial safety methods remains scarce despite their cultural importance. **Objectives.** This study aimed to evaluate the impact of Ultrasound-assisted thermal processing (USTP) on microbial safety and physicochemical properties on tejuino beverage. **Materials and Methods.** Commercial tejuino samples (200 mL) underwent ultrasonic treatment at 0.5 kJ/mL at 20 °C, followed by thermal treatment at 45-65°C (USTP– USTP 65). Fresh, pasteurized (65°C/30 min), and ultrasound-treated samples served as controls. Analyses included microbiological [aerobic mesophilic bacteria (AMB), coliform bacteria (CB), molds and yeasts (MY), lactic acid bacteria (LAB)] and physicochemical parameters (pH, acidity, soluble solids, browning index, density, viscosity, conductivity, turbidity, color), as well as antioxidants [total soluble phenols (TSP), DPPH, ABTS, FRAP]. **Results.** AMB, CB, and MY counts decreased under USTP treatments while maintaining substantial LAB concentrations in a temperature-dependent response (USTP45–USTP65) compared to controls. All physicochemical properties except density were altered by USTP treatments in a temperature-dependent manner compared to fresh control. USTP45, USTP50, and USTP55 treatments showed similar TSP content and antioxidant activity by DPPH and ABTS compared to ultrasound-treated and fresh controls, while FRAP values decreased significantly in temperatures of >50°C. **Conclusions.** The ultrasound-assisted thermal processing can be a viable alternative for the industrial manufacturing of tejuino.

RESUMEN

Introducción. El tejuino es una bebida mexicana artesanal fermentada a base de maíz con una vida útil limitada, lo que dificulta su producción industrial. A pesar de su importancia cultural, las investigaciones sobre métodos de seguridad microbiana siguen siendo escasas. **Objetivos.** El objetivo de este estudio fue evaluar el impacto del procesamiento térmico asistido por ultrasonidos (USTP) en la seguridad microbiana y las propiedades fisicoquímicas del tejuino. **Materiales y Métodos.** El tejuino comercial (200 mL) se sometió a ultrasonido (0,5 kJ/ml a 20 °C), seguido de tratamiento térmico a 45-65 °C (USTP45–USTP65). Se utilizaron muestras frescas, pasteurizadas (65 °C/30 min) y tratadas con ultrasonido como controles. Se evaluaron parámetros microbiológicos [bacterias mesófilas aeróbicas (BMA), bacterias coliformes (BC), mohos y levaduras (ML), bacterias del ácido láctico (BAL)] y fisicoquímicos (pH, acidez, sólidos solubles, índice de oscurecimiento, densidad, viscosidad, conductividad, turbidez, color), así como antioxidantes [fenoles solubles totales (PST), DPPH, ABTS, FRAP]. Resultados. Los recuentos de BMA, BC y ML disminuyeron con el USTP, con concentraciones adecuadas de BAL, efecto dependiente de la temperatura (USTP45-USTP65) al comparar con los controles. Excepto la densidad, los demás parámetros fisicoquímicos se vieron influenciados por el USTP. Los tratamientos USTP45, USTP50 y USTP55 mostraron contenido de TSP y actividad antioxidante por DPPH y ABTS similares a los controles fresco y tratado con ultrasonido, mientras que, los valores de FRAP decrementan a temperaturas >50 °C. **Conclusiones.** El procesamiento térmico asistido por ultrasonido puede ser una alternativa viable para la fabricación industrial de tejuino.

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INTRODUCTION

Mexico is home to a diverse range of traditional fermented food products, with approximately 200 such foods and beverages documented across the nation(1). These traditional fermented products are esteemed for their ritualistic, nutritional, gastronomic, and medicinal applications, and they hold significant importance in the country's economic and cultural heritage, with tejuino being particularly noteworthy(2). Tejuino is a traditional Mexican beverage made from fermented corn kernels. This staple has been central to Mexican culture since pre-Columbian times, and it is currently produced and consumed nationwide. Tejuino can be prepared using either artisanal (grain germination-fermentation) or commercial (grain nixtamalization-fermentation) methods. In both instances, fermentation occurs in non-special containers in a spontaneous and uncontrolled manner, involving lactic acid bacteria, yeast, and molds, which substantially contribute to their sensory attributes(3-5).

Typically, this refreshing beverage is offered at street stalls rather than formal establishments. These stalls consist of bicycles modified to maintain the cold temperature of the ice and tejuino concentrate(6). It is frequently consumed at gatherings, cultural rituals, and celebrations honoring Patron Saints, and it is commonly available in areas with high foot traffic, such as marketplaces, downtown areas, or outside of churches(3). Reports indicate that tejuino consumption in Mexico averages 2 L per person per week(3). Moreover, some studies classify tejuino as a functional beverage due to its content of various polysaccharides with prebiotic benefits and a diversity of microorganisms with probiotic effects(3,5,7). However, the presence of deteriorative and pathogenic microorganisms has been reported in commercial tejuino(3, 7-8). Additionally, artisanal or commercial tejuino lacks preservatives, resulting in a reduced shelf life, which poses a challenge for industrial production. Although tejuino holds traditional and cultural importance, there is limited research on using thermal or non-thermal strategies to extend its shelf life and ensure its microbial safety.

Conventional thermal pasteurization remains the most prevalent method for extending the shelf life of solid, viscous, and liquid food products, including fermented items. This technique effectively inactivates enzymes, non-spore-forming pathogenic bacteria, and most vegetative spoilage microorganisms(9). On the other hand, the application of heat treatment to fermented beverages can result in the degradation of nutritional components and induce browning reactions, thereby altering their sensory attributes, such as color, flavor, and texture(10). Nonetheless, thermal treatments can be integrated with other preservation technologies, such as ultrasound, to apply thermal treatments at low temperatures.

High-intensity ultrasound (HIUS) is an innovative food-processing technique employed to deactivate enzymes and eliminate pathogenic and spoilage microorganisms, thereby extending the shelf life of various food products. This method transmits sound waves at frequencies above the human hearing range (20–40 kHz) via a liquid medium(11). The core mechanism of HIUS is acoustic cavitation, which induces physical, mechanical, and chemical effects through the rapid collapse of microbubbles and the release of energy, leading to the inactivation of microorganisms(12). Several studies have investigated using ultrasound to regulate or enhance microbial activity in milk-based fermented drinks(10-12). Moreover, ultrasound can be applied alone and in combination with heat, although when ultrasound is combined with heat, it is usually applied simultaneously (called thermosonication)(13). In contrast, HIUS can be used as a pretreatment before thermal treatment, in which HIUS can enhance the rate of food pasteurization and reduce the duration and intensity of thermal treatment without affecting food quality. Ultrasound-assisted thermal processing is a strategy that causes minimal loss of sensory and nutritional characteristics(14).

HIUS treatment applied with or without heat has been investigated as an alternative to conventional homogenization and heat treatment for milk-based beverages(10) , fermented whey and oat beverages(15), fermented milk(11), yogurt(16), and chocolate milk beverages(12). However, there are no reports in the literature regarding the use of ultrasound-assisted thermal processing of tejuino. Therefore, this study aimed to evaluate the effect of ultrasound-assisted thermal processing on microbial stability and physicochemical properties of tejuino beverage.

MATERIALES Y MÉTODOS

Commercial Tejuino

Ten liters of commercial tejuino were acquired from a local store in Yahualica, Jalisco, Mexico. The samples were transported to the Microbiology Food Laboratory of the Centro Universitario de Los Altos from the University of Guadalajara, under refrigerated conditions, for further analysis. For each treatment (**Table 1**), 200 mL of tejuino was placed in a 250 mL glass beaker (previously sterilized at 121 °C for 15 min).

Table 1. Experimental matrix for ultrasonic pretreatment and low thermal treatment of Tejuino

Sample	Ultrasonic pretreatment (KJ/mL)	Thermal treatment	
		Temperature (°C)	Time (min)
Fresh control	-	-	-
Pasteurized control	-	65	30
Ultrasound control	0.5	-	-
USTP45	0.5	45	30
USTP50	0.5	50	30
USTP55	0.5	55	30
USTP60	0.5	60	30
USTP65	0.5	65	30

Ultrasonic pretreatment

Tejuino samples underwent ultrasonic treatment at an energy level of 0.5 kJ/mL [selected according to previous reports in milk(17)] maintained at a constant temperature of 20 °C, followed by the application of the corresponding thermal treatment as detailed in **Table 1.** This process utilized a high-intensity ultrasonic probe processor (Model PZ-550LI, Fangxu Technology Co., Ltd, China) which has a nominal output power of 550 watts and operates at a frequency of 24 kHz. The ultrasonic processor was equipped with a 6 mm diameter ultrasonic probe (T1-6AL-4V) and delivered 25 watts of real ultrasonic energy, working at an amplitude of 100 µm. The energy density applied during the ultrasonic pretreatment was achieved using a continuous ultrasound residence time of 66.67 minutes, as calculated by Solano-Cornejo & Rojas(17) using equation (1).

$$t=\frac{V \text{ x } E \text{ x } 1000}{P \text{ x } 60}$$

where: t = Treatment time (min); V = tejuino volume (200 mL); E = Expected energy density (0.5 kJ/mL); P = Real acoustic power (25 W).

Before ultrasound processing, the ultrasound sonotrode and the ultrasound chamber were cleaned and disinfected with a 70% ethanol solution before treatment. Additionally, the ultrasound chamber was kept closed during processing.

Thermal treatment of ultrasonically pretreated Tejuino

After ultrasonic treatment, the tejuino samples underwent thermal treatment in a water bath (Thermo Scientific 2870, Ohio, USA) at 45, 50, 55, 60, and 65 °C for 30 minutes, as detailed in (**Table 1**). Once the thermal process was complete, the flasks were removed from the water bath and immediately placed in an ice bath. They were then stored at 4°C for up to 3 hours before analysis(17). To avoid cross-contamination, the tejuino sample (in a glass beaker) was covered with aluminum foil during thermal treatment.

Microbiological analysis

For microbial analysis, decimal dilutions up to 10^7 were prepared to evaluate aerobic mesophiles, coliforms, lactic acid bacteria, mold, and yeasts using the pour-plate method. For this, ten milliliters of the sample were collected after thermal treatment. The sample was subjected to a 10-fold serial dilution in sterile peptone solution at 0.1% (casein peptone: 1 g/L and sodium chloride: 8.5 g/L), and the appropriate dilution (1 mL) was inoculated onto selective agar media. Aerobic mesophilic bacteria (AMB) were counted using plate count agar (Sigma Aldrich, USA) at 35 °C for 48 h(18), while total coliform bacteria (TCB) were evaluated on violet-red bile glucose agar (BD DifcoTM, France) at 35 °C for 24 h(19); molds and yeast were quantified on potato dextrose agar (Sigma Aldrich, USA) acidified at 5.6 pH with a 10% tartaric acid solution and incubated at 25 °C for 120 h(20). Lactic acid bacteria (LAB) were identified on de Man, Rogosa, and Sharpe agar (BD DifcoTM, France) and incubated at 37 °C for 48 h in anaerobic conditions(21). Results were reported as log CFU/mL of tejuino. The culture media were prepared following the manufacturer's instructions. All reagents and materials used here were sterilized at 121 °C for 15 min, and microbial analysis was performed in a laminar flow hood.

Physicochemical parameters

The assessment of total soluble solids (TSS) was carried out using a portable refractometer (PAL87S, Atago Co., Ltd., Tokyo, Japan), with the results presented in °Brix(22). Titratable acidity was measured using the titration-pH method, where 5 mL of the sample was mixed with 25 mL of distilled water and titrated with 0.1 N NaOH until reaching a pH of 8.30, with the results expressed as a percentage of lactic acid (23). The pH was determined with a pH meter (Hanna HI 207; Bedford, UK)(22). The electrical conductivity (EC) was determined with a portable conductivity meter (Hanna Instruments HI 8733, Bedford, UK), and results are reported in mS/cm following the manufacturer's instructions. Viscosity was evaluated using a Digital Viscometer (Brookfield DV2T HB, Middleboro, USA) at 30 rpm and using the spindle #02, with the results expressed in centipoise (24). The turbidity of tejuino was measured at room temperature using a HACH 2100N Turbidimeter (Loveland, Colorado, USA) with sample cells measuring 25 mm in diameter and 95 mm in height, following the manufacturer's guidelines. The results were reported in Nephelometric Turbidity Units (NTU). A dilution factor of 1:2 (sample: water) was used to measure the turbidity. The non-enzymatic browning index (NEBI) was determined spectrophotometrically (UV-5100PC, Shangai Metash Instruments CO, LTD, China) at 420 nm following the method of Cohen et al., (25). Color analysis was conducted using a portable colorimeter (FRU, WR10QC, Shenzhen, China), with the findings reported on the CIELab* scale (luminosity, a*, and b*) and total color difference (TCD) was calculated using equation (2)(26).

$$\mathrm{TCD} = \sqrt{(L-L_0)^2+(a-a_0)^2+(b-b_0)^2}$$

Where L, a*, and b* are the color values of fresh tejuino, whereas L0, a0 and b0 are color values of USTP-treated tejuino.

Quantification of soluble phenols and antioxidant activity

To quantify soluble phenols (TSP) and antioxidant activity (assessed by DPPH, ABTS, and FRAP assays), an aqueous–organic extraction was conducted by mixing 2 mL of Tejuino with 10 mL of acidified methanol-water solution (80:20 v/v plus 2% HCl 2 M)(27).

For TSP quantification, 12 µL of the sample extract, 12 µL of Folin–Ciocalteu reagent 2 N (Sigma-Aldrich, USA), 116 µL of 7.5% w/v Na_2CO_3, and 164 µL of distilled water were mixed in a tube test and incubated in darkness for 15 min. After this period, 200 µL of the mixture was placed in a 96-well plate, and the absorbance was read in a plate reader at 750 nm (ACCURIS Instruments, SmartReader MR-9600, Nankín, China)(28). A calibration curve (R^2 = 0.998) of gallic acid (Sigma-Aldrich, USA, purity >98%) was constructed, and the results were expressed as mg equivalents of gallic acid/100 mL (mg GAE/100 mL).

To evaluate the scavenging activity of the DPPH radical, a 96-well microplate assay was performed. It involved mixing 260 µL of DPPH solution (190 µM) (Sigma-Aldrich, USA, purity of 97%) with 40 µL of extract, followed by incubation with shaking (200 rpm) for 30 minutes in the dark. The absorbance was then recorded at 517 nm using a microplate reader(29). A calibration curve with an R^2 of 0.992 was prepared using Trolox standard (Sigma-Aldrich, USA, purity of 97%), and the scavenging capacity was expressed as millimoles of Trolox equivalents/100 mL (mmol TE/100 mL).

To assess ABTS•+ radical scavenging activity, 265 µL of ABTS•+ solution (Sigma-Aldrich, USA, purity >98%) was mixed with 35 µL of extract in a 96-well microplate. The mixture was incubated with shaking at 200 rpm for 10 minutes in the dark. Afterwards, absorbance was measured at 734 nm using a microplate reader(30). ABTS (7 mM) was stirred magnetically overnight (16 h) in potassium persulfate (2.45 mM) and then diluted with phosphate buffer (pH 7.4) to an absorbance of 0.7±0.02 at 734 in a UV-Vis spectrophotometer (UV-5100PC, Shangai Metash Instruments CO, LTD, China). A calibration curve with Trolox standard (Sigma-Aldrich, USA) ($R^2 = 0.999$) was generated, and the results were expressed as millimoles Trolox equivalents/100 mL (mmol TE/100 mL).

For the FRAP assay, a mixture was prepared by combining 36 µL of the extract, 264 µL of FRAP solution, and 9 µL of distilled water in a test tube. The mixture was stirred at 200 rpm in the dark for 30 minutes. Afterwards, 200 µL of the mixture was transferred to a 96-well plate, and the absorbance was measured at 595 nm using a microplate reader (31). A calibration curve ($R^2 = 0.997$) was constructed with the Trolox standard (Sigma-Aldrich, USA), and the results were expressed as millimoles Trolox equivalent/100 mL (mmol TE/100 mL). The FRAP solution containing TPTZ (Sigma-Aldrich, USA, purity >98%), FeCl3 (FAGA, Mexico, purity of 97%), and acetate buffer composed of sodium acetate anhydrous (Sigma-Aldrich, USA, purity >99%) and glacial acetic acid (Fermont, Mexico, purity of 99.8%) was prepared as recommended.

Statistical analysis

All experiments were conducted in triplicate and reported as mean ± SD. One-way ANOVA was used, with Tukey's test for post-hoc analysis ($p<0.05$). Principal component analysis was employed to examine correlations among all variables and evaluate the relationships between treatments, considering physicochemical and antioxidant parameters. All data analyses were carried out using Statistica software (version 10, StatSoft, Tulsa, OK, USA).

RESULTS

Figure 1 illustrates the visual appearance of tejuino before and after undergoing ultrasound and thermal pretreatments.

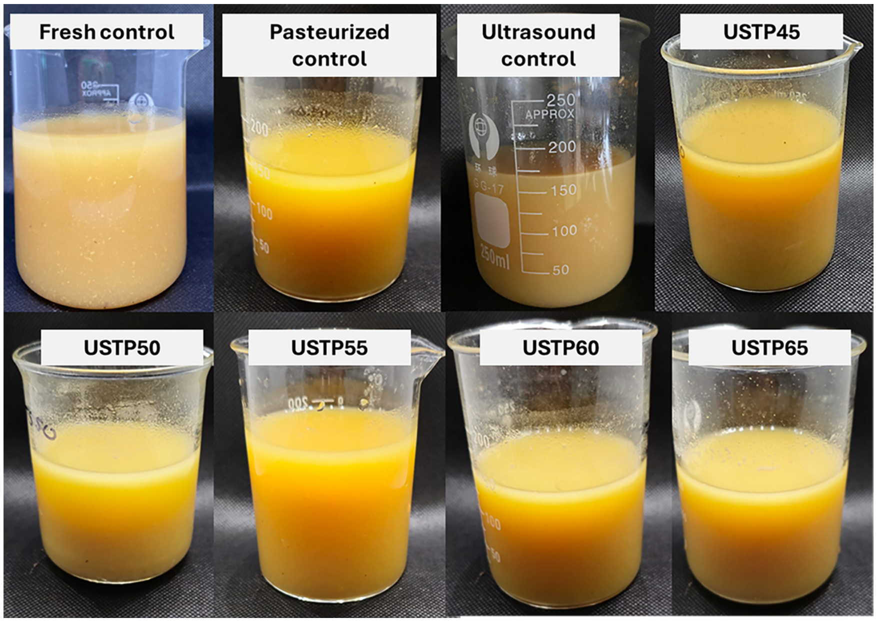

Figure 1. Visual appearance of Tejuino beverage before and after treatments.

Table 2 shows the microbial counts of tejuino following ultrasound-assisted thermal processing (USTP) at varying temperatures. The fresh control exhibited significantly ($p<0.05$) higher AMB (8.25 log CFU/mL), CB (7.25 log CFU/mL), molds (1.79 log CFU/mL), yeasts (2.51 log CFU/mL), and LAB (8.31 CFU/mL) counts compared to both the pasteurized (1.44, 1.91, undetectable, undetectable, and 2.58 log CFU/mL, respectively) and ultrasound-treated (5.73, 6.69, undetectable, undetectable, and 7.15 log CFU/mL) controls. However, when USTP was applied, microbial counts (AMB, CB, molds, yeasts, and LAB) were significantly reduced ($p<0.05$) compared to ultrasonic treatment alone, and in some instances, were comparable to those observed in the pasteurized control, in a temperature-dependent manner. The AMB counts of USTP treatments ranged from 4.47 log CFU/mL to undetectable as the thermal treatment temperature increased from 45 to 65°C following ultrasound pretreatment. In comparison, CB counts decreased from 4.26 log CFU/mL to undetectable levels. Additionally, molds were not detected under any USTP treatment, whereas yeasts were only quantified in USTP45 (2.43 log CFU/mL) and USTP50 (0.93 log CFU/mL) treatments. The LAB counts ranged from 6.61 to undetectable.

Table 2. Microbial counts of Tejuino after ultrasonic pretreatment and thermally treated at different temperatures

Treatment	Microorganism (Log CFU/mL)				
	Aerobic mesophilic bacteria	Coliform bacteria	Molds	Yeast	Lactic acid
Fresh control	8.25 ± 0.01a	7.25 ± 0.02a	1.79 ± 0.06	2.51 ± 0.02a	8.31 ± 0.03a
Pasteurized control	1.44 ± 0.37e	1.91 ± 0.04e	ND	ND	2.58 ± 0.39f
Ultrasound control	5.73 ± 0.02b	6.69 ± 0.01b	ND	2.43 ± 0.04a	7.15 ± 0.02b
USTP45	4.77 ± 0.02f	4.26 ± 0.03c	ND	0.93 ± 0.48b	6.61 ± 0.01c
USTP50	4.83 ± 0.01f	2.83 ± 0.01d	ND	ND	6.72 ± 0.03c
USTP55	3.22 ± 0.01c	1.51 ± 0.17f	ND	ND	5.65 ± 0.04d
USTP60	2.20 ± 0.03d	ND	ND	ND	5.15 ± 0.01e
USTP65	ND	ND	ND	ND	ND

The data are presented as means ±SD (n = 3). Statistically significant differences among treatments within each column are indicated by different letters (α = 0.05). ND: Not detected.

Table 3 presents the pH, titratable acidity, total soluble solids, and non-enzymatic browning index (NEBI) values for both untreated and treated tejuino samples. Significant differences ($p< 0.05$) were observed among the treatments for all parameters evaluated. In terms of pH values, no differences were detected among treatments (4.08 – 4.11, $p>0.05$). Regarding TA, the fresh (1.53%) and ultrasound-treated (1.57%) controls showed comparable values, as did the USTP45, USTP50, and USTP55 treatments (1.55–1.58%; $p>0.05$). However, a significant increase ($p<0.05$) in titratable acidity was noted in the pasteurized control (1.63%) and in USTP samples at higher temperatures, including USTP60 (1.64%) and USTP65 (1.60%), relative to the fresh and ultrasound-treated controls. Regarding total soluble solids, an increase was observed in the pasteurized (7.16 °Brix), and USTP samples (7.22–7.75 °Brix), irrespective of the temperature applied in these treatments (USTP45–USTP65), when compared to the fresh and ultrasound-treated controls (6.47 and 6.57 °Brix). In terms of NEBI values, a decrease was observed in the ultrasound-treated (0.22) and USTP samples (0.19–0.22), regardless of the temperature used (USTP45–USTP65), in comparison with the fresh (0.33) and pasteurized (0.29) controls.

Table 3. pH, titratable acidity, total soluble solids, and NEBI values of Tejuino under ultrasonic pretreatment thermal treated at different temperatures

Treatment	pH	Titratable acidity (% lactic acid)	Total soluble solids (°Brix)	NEBI
Fresh control	4.08 ± 0.01a	1.53 ± 0.05a	6.47 ± 0.01d	0.33 ± 0.01a
Pasteurized control	4.10 ± 0.01a	1.65 ± 0.01b	7.16 ± 0.16c	0.29 ± 0.01b
Ultrasound control	4.09 ± 0.03a	1.57 ± 0.07a	6.57 ± 0.19d	0.22 ± 0.01f
USTP45	4.11 ± 0.01a	1.58 ± 0.01a	7.22 ± 0.25bc	0.21 ± 0.01cf
USTP50	4.10 ± 0.01a	1.57 ± 0.06a	7.66 ± 0.16bc	0.20 ± 0.01de
USTP55	4.09 ± 0.01a	1.55 ± 0.01a	7.57 ± 0.09bc	0.21 ± 0.01cd
USTP60	4.11 ± 0.01a	1.64 ± 0.01b	7.75 ± 0.16a	0.19 ± 0.01e
USTP65	4.12 ± 0.01a	1.60 ± 0.01b	7.75 ± 0.22a	0.22 ± 0.01f

All values are presented as means ± SD (n = 3). Distinct letters within each column denote statistically significant differences between treatments (α = 0.05). NEBI: non-enzymatic browning index.

Table 4 shows the density, viscosity, electrical conductivity, and turbidity values of both the untreated and treated tejuino samples. The application of USTP treatments did not result in significant changes in density (1.02 g/L; $p>0.05$). A significant reduction ($p<0.05$) in viscosity was noted in both pasteurized (315 cP) and ultrasound-treated (290 cP) controls, as well as in USTP samples (189–205 cP), exhibiting a temperature-dependent response (USTP45–USTP65) when compared to the fresh control (413 cP). Conversely, an increase ($p<0.05$) in the electrical conductivity was observed across all treated samples (1.59–1.69 mS/cm) relative to the fresh control (1.31 mS/cm). A similar pattern was observed for turbidity, with all treatments resulting in a significant increase ($p<0.05$) in turbidity among all treated samples (5533–9503 NTU) compared with the fresh control (4778 NTU).

Table 4. Density, viscosity, electrical conductivity, and turbidity values of Tejuino under ultrasonic pretreatment, thermal treated at different temperatures

Treatment	Density (g/L)	Viscosity (cP)	Turbidity (NTU)	Electrical conductivity (mS/cm)
Fresh control	1.02 ± 0.01a	413 ± 2.02a	4778 ± 30f	1.51 ± 0.01d
Pasteurized control	1.02 ± 0.01a	315 ± 1.01b	5533 ± 44e	1.69 ± 0.01a
Ultrasound control	1.02 ± 0.01a	290 ± 7.00c	6749 ± 6d	1.59 ± 0.01c
USTP45	1.02 ± 0.01a	189 ± 1.20f	9503 ± 13a	1.68 ± 0.01a
USTP50	1.02 ± 0.01a	164 ± 5.00e	9194 ± 67g	1.63 ± 0.03b
USTP55	1.02 ± 0.01a	205 ± 5.10d	9259 ± 20g	1.66 ± 0.01ab
USTP60	1.02 ± 0.01a	205 ± 7.10f	8514 ± 44b	1.67 ± 0.01a
USTP65	1.02 ± 0.01a	193 ± 3.10f	7640 ± 34c	1.67 ± 0.01a

Data are presented as mean ± standard deviation (n = 3). Statistically significant differences among treatments within each column are indicated by different letters ($p<0.05$).

Table 5 lists the color attributes (luminosity and a* and b* coordinates) and total color difference between the untreated and treated tejuino samples. All color parameters are influenced by the treatments ($p<0.05$). Thermal treatment (pasteurized control) promoted an increase in luminosity (36.56) compared to the fresh (32.26) and ultrasound-treated (30.83) controls. Moreover, a tendency to increase the luminosity values can be observed for all USTP samples (31.63–35.17) in a temperature-dependent manner. Furthermore, all treated samples showed significant differences in a* and b* values compared to the fresh control. Also, the pasteurized sample exhibited a higher TCD value (4.81) than the ultrasound-treated control (1.85). In contrast, the USTP samples exhibited a TCD ranging from 1.66 to 3.01, with a tendency to increase with increasing temperature.

Table 5. Color attributes of Tejuino under ultrasonic pretreatment and thermal treated at different temperatures

Treatment	Luminosity	a*	b*	TCD
Fresh control	32.26 ± 0.25de	0.94 ± 0.01f	10.92 ± 0.28bcd	-
Pasteurized control	36.56 ± 0.43b	-1.19 ± 0.45a	10.53 ± 0.82cd	4.81
Ultrasound control	30.83 ± 0.61e	0.27 ± 0.20cd	9.93 ± 0.17d	1.85
USTP45	31.63 ± 1.26de	0.39 ± 0.04bc	12.52 ± 0.71a	1.81
USTP50	32.79 ± 0.45ad	-0.42 ± 0.02e	11.72 ± 0.20ab	1.66
USTP55	34.96 ± 0.53bc	-0.14 ± 0.04de	10.28 ± 0.01d	2.97
USTP60	34.35 ± 0.23ac	0.87 ± 0.02bf	11.57 ± 0.10abc	2.18
USTP65	35.27 ± 0.47bc	0.97 ± 0.06f	11.07 ± 0.13bcd	3.01

Data are shown as mean ± SD (n = 3). Significant differences among treatments in each column are marked by different letters ($p< 0.05$). The parameter a* indicates the red/green coordinate, b* the yellow/blue coordinate, and TCD represents the total color difference.

Table 6 shows the total soluble phenols (TSP) content and antioxidant capacity (DPPH, ABTS, and FRAP) of fresh, pasteurized, ultrasound-treated, and USTP samples. Significant differences ($p<0.05$) among treatments were observed for all parameters evaluated. The application of ultrasound alone (119.64 mg GAE/100 mL) significantly increased the TSP content compared to fresh and pasteurized (93.76 and 82.10 mg GAE/100 mL) controls. Moreover, the USTP45 to USTP60 exhibited similar TSP contents (87.04 – 104.82 mg GAE/100 mL) to ultrasound-treated and fresh controls, where USTP65 and pasteurized control exhibited a reduction in TSP content (82.10 and 76.99 mg GAE/100 mL) compared to the fresh control.

Regarding antioxidant activity, the application of USTP45 – USTP65 promotes a decrease in DPPH (101 – 136 mmol TE/100 mL), ABTS (100.14 – 145.08 mmol TE/100 mL), and FRAP (43.75 - 97.16 mmol TE/100 mL) values in a temperature-dependent response in comparison with the fresh control (143.52, 146.95, and 96.51 mmol TE/100 mL, respectively), where the lowest values were observed at USTP65, which were similar to those observed in the pasteurized control (98.84, 77.13, and 18.43 mmol TE/100 mL, respectively).

Table 6. Total soluble phenols content and antioxidant activity of Tejuino under ultrasonic pretreatment and thermal treatment at different temperatures

Treatment	Total soluble phenols (mg GAE/100 mL)	Antioxidant activity (mmol TE/100 mL)		
		DPPH	ABTS	FRAP
Fresh control	93.76 ± 0.85cd	143.52 ± 0.51b	146.95 ± 0.06a	96.51 ± 0.88b
Pasteurized control	82.10 ± 3.20e	98.84 ± 1.46e	77.13 ± 2.50e	18.43 ± 1.10g
Ultrasound control	119.64 ± 12.09a	153.28 ± 0.62a	147.09 ± 0.10a	98.70 ± 0.48a
USTP45	104.82 ± 8.05b	136.44 ± 0.51c	145.08 ± 0.15a	97.18 ± 1.07b
USTP50	98.30 ± 5.60bc	133.60 ± 2.49c	144.87 ± 0.10a	81.07 ± 0.38c
USTP55	93.56 ± 2.26cd	135.56 ± 0.70c	141.44 ± 0.10b	79.46 ± 0.37d
USTP60	87.04 ± 3.07de	111.28 ± 3.04d	122.63 ± 0.24c	62.27 ± 0.76e
USTP65	76.99 ± 2.47e	101.32 ± 3.81e	100.14 ± 4.50d	43.75 ± 0.33f

Statistically significant differences between treatments ($p < 0.05$) are indicated by different letters in each column.
GAE: Gallic acid equivalent; TE: Trolox equivalent.

Principal component analysis (PCA) was employed to explore the relationship between the physicochemical characteristics of tejuino and the treatments under USTP (**Figure 2**). The PCA identified seven principal components, but only the first two were considered due to their eigenvalues (PC1:7.07 and PC2:4.19), which accounted >75% of the total variance (PC1:47.16% and PC2:27.96%). **Figure 2a** illustrates the graph of tejuino's physicochemical properties and antioxidants, while (**Figure 2b**), displays the positions of the evaluated treatments. In a scatter plot of the score values for all physicochemical parameters and antioxidants projected in the PC1 plane, EC (0.86), luminosity (0.81), TA (0.81), pH (0.79), TSS (0.76), and density (0.63) tends to increase, whereas DPPH (-0.93), FRAP (-0.86), ABTS (-0.82), and TSP (-0.74) decreased, proceeding from positive to negative. Additionally, in PC2, turbidity (0.93) increased, while NEBI (-0.88) and viscosity (-0.83) decreased, proceeding from positive to negative (**Figure 2a**). PCA differentiated between fresh, pasteurized, and ultrasonicated controls and USTP treatments (**Figure 2b**). Based on the factor coordinates (from negative to positive in PC1), the fresh control (-3.95) and ultrasound control (-3.32) were similar. USTP55 (-0.34), USTP50 (-0.24), and USTP45 (-0.08) were more closely related to each other, whereas USTP60 (1.81) differed from all treatments. Conversely, USTP65 (3.05) and the pasteurized control (3.11) showed marked differences from the fresh and ultrasound controls and USTP45-USTP60 treatments.

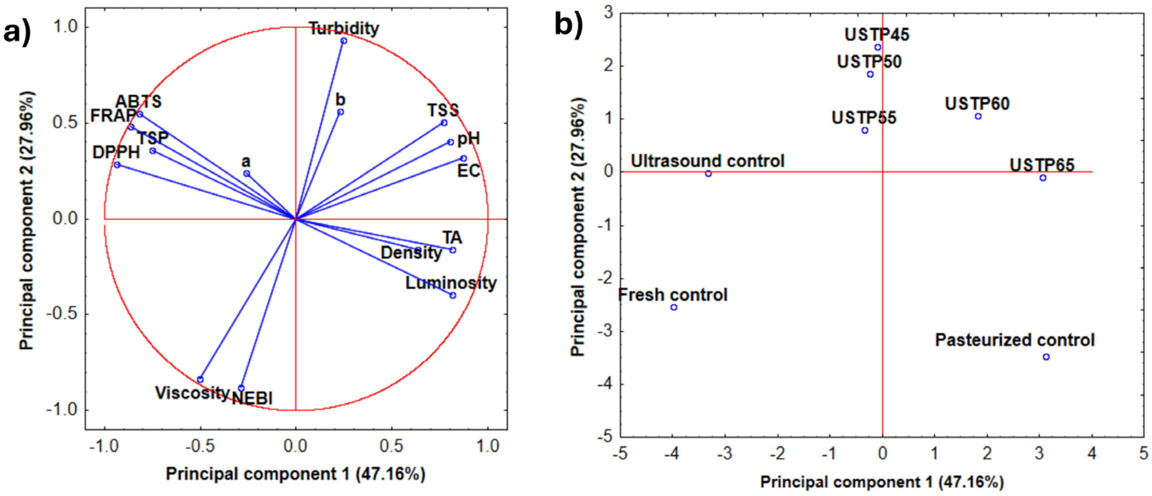

Figure 2. Principal component plots of tejuino samples. Location of physicochemical and antioxidants parameters **(a)** and location of treatments **(b)**. **TSS**: total soluble solids, **EC**: electrical conductivity, **TA**: titratable acidity, **NEBI**: non-enzymatic browning index, **TSP**: total soluble phenols, **USTP**: ultrasound-assisted thermal processing.

DISCUSSION

Non-alcoholic fermented foods have been reported to exhibit a limited shelf-life post-fermentation. This is attributed to the presence of lactic acid bacteria, and potential presence of molds, yeasts, and other spoilage microorganisms(10). The microbial counts in fresh tejuino were similar than that reported in the study by Rubio-Castillo et al.(7) for AMB (7.17 – 8.66 log CFU/mL) and LAB (6.98 – 8.28 log CFU/mL) and lower for MY (4.74 – 8.47 log CFU/mL), also they reported the absence of coliform bacteria. These differences may be attributable to the elaboration process. For its part, pasteurization is an effective thermal treatment applied to reduce microbial counts in a variety of beverages, including fermented beverages; however, it has been reported that this treatment significantly affects the nutritional and sensorial properties of treated products(9). Regarding high-intensity ultrasound treatment, it can reduce spoilage microorganisms in beverages by cavitation phenomena. Still, its effect for inactivating microorganisms is limited and influenced by many factors such as viscosity, soluble solids, and concentration and type of microorganisms(32).

For this reason, ultrasound is typically combined with thermal treatments, applied simultaneously or as pretreatment to accelerate the rate of microbial inactivation, in which ultrasound promotes higher cell sensitivity by cavitation and thermal treatment promotes localized heating, thus leading to cell death(33). In this study, under USTP treatments, there was a notable decrease in AMB, CB, and MY counts, mainly in USTP45, USTP50, USTP55, and USTP60, while a significant level of lactic acid bacteria was maintained. Some microorganisms are more resistant than others to USTP, or shape, size, concentration, and cell sensitivity of these microorganisms, and experimental conditions can influence treatment efficiency(13). It has been reported that AMB and CB populations in milk pre-sonicated at 0.5 kJ/mL responded variably to the temperatures tested (40 to 60 °C), showing growth in the range of 40 to 50 °C, no growth at 50 °C, and inactivation at 60 °C. Nonetheless, an AMB and BC count increase was observed in USTP treatments under 50°C(34). It has been reported that there is a significant decrease in AMB counts after USTP60 in fermented milk beverage (10). Regarding BAL, it has been reported that some LAB strains exhibited thermotolerance due to the presence of heat-shock proteins; moreover, ultrasound is widely used as a pretreatment to promote the growth of LAB for fermented food production(15, 35-36). Moreover, no mold and yeast counts were detected in USTP treatments above 50°C, possibly associated with the lower initial counts of these microorganisms. It has been shown that yeast strains are more sensitive than lactic acid bacteria in ultrasound-treated wine(37).

The pH and TA values observed in fresh tejuino in this study are higher than those reported in artisanal and commercial tejuino, where differences may be attributed to the fermentation process involved in the elaboration of tejuino(7). Some authors report no changes in pH values after ultrasound plus heat treatments in fruit juices(13). It has been reported that ultrasound, pasteurization, or USTP did not promote changes in pH values in beer(38). Moreover, the increase of titratable acidity by USTP treatments may be attributed to the possible formation of acids by breaking the peptide bond of amino acids during sonication and thermal treatment, and the presence of organic acids and polyphenols, as well as the formation of specific chemical products through the sonolysis (OH^-, H^+, and H_2O_2) of tejuino by cavitation, which can contribute to the titratable acidity increase(17, 39-40). On the other hand, no pH and titratable acidity changes were observed in ultrasound-heat-treated fermented milk beverage(10). Conversely, it has been reported that there is a decrease in titratable acidity values on tomato paste after high-intensity ultrasound pretreatment(41). The increase in total soluble solids in USTP treatments compared to fresh control could be associated with thermal treatment, which can evaporate water and concentrate solids, predominantly reducing and non-reducing sugars, because the application of ultrasound in fruit-based beverages typically did not promote changes in TSS(13). It has been no changes in total soluble solids content in kiwi juice after ultrasound treatment(42). Furthermore, the USTP treatments may reduce the oxidation of tejuino promoted by non-enzymatic reactions(43).

The viscosity of tejuino is closer to that of soursop nectar(44). In tejuino samples treated with USTP, viscosity generally decreases while turbidity rises compared to fresh control. It has been observed that when ultrasound and heat are applied simultaneously to fruit beverages, cavitation reduces suspended particles (mainly soluble fibers), and temperature dissolves them (often pectin particles), leading to increased turbidity. This effect is enhanced by high sugar concentrations (45-46). Tejuino is characterized by its carbohydrate content (49.35 to 79.32%) due to the addition of brown sugar during tejuino preparation(7). Moreover, the stability of tejuino colloidal suspension after USTP may be favored by the presence of some ions (Na+, Ca+, K+, and Mg+), avoiding particle sedimentation(47). Moreover, the increase of electrical conductivity could be associated with the release of minerals from the food matrix to the medium(45). Color is a quality attribute in fermented beverages. USTP promoted slightly changes in color attributes (luminosity, a*, and b*). Similar trends were reported in kiwi juice after the application of ultrasound and heat at 45, 50, and 55°C(42). These changes may be attributed to the reduction of particle sizes, promoting changes in the light reflection(17). For its part, only the pasteurized and USTP65 exhibited TCD >3, indicating that tejuino color is very distinct from the fresh sample, while most of the USTP samples showed subtle color changes (1.5 < TCD < 3), which could be associated with the application of high temperatures(48) or release of colored compounds due to the ultrasound treatment(13).

When USTP was conducted at temperatures ≥60 °C, there was a reduction in total soluble phenols content. Initially, ultrasound may release phenolic compounds from the food matrix due to cell wall breakdown caused by cavitation during ultrasound treatment, as demonstrated after applying USTP at 45 °C and 50 °C to kiwi fruit (42). However, some hypotheses regarding the degradation/oxidation of phenolic compounds suggest that after ultrasound release, it may be degraded by free radicals generated by the water sonolysis during cavitation process. Consequently, antioxidants interact with these free radicals, thereby reducing their concentration(40). Furthermore, it has been documented that thermal processing can notably lower the total soluble phenols levels in pomegranate juice, attributed to their degradation or oxidation(26). In this context, a decrease in the antioxidant activity by DPPH, ABTS, and FRAP was detected in USTP treatments in a temperature-dependent manner. This effect can be attributed to the reduction of soluble phenols and other antioxidant compounds during ultrasound-assisted thermal processing(49). Nonetheless, USTP-treated tejuino could be a source of antioxidants; in this sense, studies on the colonic fermentation of tejuino's indigestible components have suggested that this fermented beverage could be a potential source of metabolites with antioxidant properties(50).

CONCLUSIONS

The application of ultrasound-assisted thermal processing (USTP) on tejuino effectively reduces the microbial populations of aerobic mesophilic bacteria, coliform bacteria, molds, and yeasts, while maintaining substantial levels of lactic acid bacteria in a temperature-dependent manner. Except for density, the physicochemical properties of tejuino were only slightly affected by USTP treatments. Furthermore, USTP at lower thermal temperatures (45-55 °C) preserved the total soluble phenols and antioxidant activity (DPPH and ABTS) at levels comparable to those of fresh tejuino. To the best of our knowledge, this is the first report on the use of ultrasound-assisted thermal processing for tejuino beverages. The implementation of USTP presents a viable alternative for processing tejuino. However, further research is necessary to evaluate the effects of USTP at different ultrasonic energy levels on the kinetic inactivation of spoilage and pathogenic bacteria, as well as on physicochemical parameters, and to assess the effect of USTP during cold storage. Moreover, it is essential to assess the overall acceptability of USTP-tejuino among consumers through sensory tests.

ACKNOWLEDGMENTS

Thanks to Tatiana Lizbeth Pérez Santivañez, Andrea Quetzalli Uribe Torres, and Gerardo Aceves Alcala for technical support as part of their "Early Incorporation into Research Program" activities from the Centro Universitario de Los Altos (CUALTOS) of the University of Guadalajara, and to the Instituto de Investigación en Ciencias Médicas for the use of infrastructure from the CUALTOS.

FUNDING

This research received no external funding.

ETHICAL CONSIDERATIONS

This study did not involve human participants, animals, or personal data. The artisanal samples analyzed were commercially acquired. Therefore, ethical approval and informed consent were not required. All laboratory procedures were conducted following applicable biosafety and biohazard waste management regulations.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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