

Article

Effect of Pretreatment with Low-Frequency Ultrasound on Quality Parameters in Gulupa (*Passiflora edulis* Sims) Pulp

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Featured Application: Ultrasound treatment offers a new strategy to improve and extend the shelf life in minimally processed fresh fruit.

Abstract: The Gulupa (*Passiflora edulis* f. *edulis* Sims) is an expression of South America's tropics' biodiversity, and a source of B vitamins and amino acids. It is a climacteric export fruit for which it is necessary to incorporate emerging technologies for its conservation and transport. This work investigated the effect of ultrasound on gulupa pulp and verified the stability of the characters of interest in the shelf life of 20 days. Six treatments and a control sample were used, evaluated in triplicate, and varied in frequency (30 and 40 kHz) with an exposure time of 10, 20, and 30 min. A statistical analysis of unidirectional variances and Dunnett's test was used. It was found that the ultrasound treatments did not affect the pH or the titratable acidity. Soluble solid results presented a significant increase ($p < 0.05$) (from 13.4 to 14.8% *w/v*) in the antioxidant capacity (from 1.13 to 1.54 μmol Trolox Equivalent (TE)/g by the ABTS•+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) Cationic Radical Assay and from 3.3 to 3.7 μmol TE/g by the DPPH• (2,2-diphenyl-1-picrylhydrazil) Radical Scavenging Assay). During the shelf life, ascorbic acid was the parameter that varied most ($p < 0.05$). It decreased from 42.7 to 21.6 mg ascorbic acid/100 g of pulp in the control sample. However, a smaller decrease was observed (23.8–24.5 mg ascorbic acid/100 g of pulp) in the 40 kHz treatments. The smallest global color difference (ΔE) for the control was found in the 40 kHz treatment at 30 min through the entire shelf life (day 0 to 20). Ultrasound treatment offers a new strategy to improve and extend the shelf life of chilled gulupa pulp.

Keywords: antioxidant capacity; biomolecules; conservation; postharvest; pulp; shelf life

1. Introduction

Gulupa (*P. edulis* f. *edulis*), also known as purple passion fruit, of the Passifloraceae family, is a climbing plant native to southern Brazil, Paraguay, and northern Argentina. It

is produced mainly in Brazil, followed by Venezuela, Colombia, and the rest of the tropical countries [1], from where it is mainly exported to the European Union. Colombia has the most remarkable diversity of the genus *Passiflora*, among which *P. edulis* f. *flavicarpa* (yellow passion fruit), *P. edulis* f. *edulis* (purple passion fruit or gulupa), *P. ligularis* (sweet granadilla or grenadia), and *P. mollissima* (banana passion fruit or curuba) are the most desired in international markets. In 2019, Colombia exported 8725 tons of Gulupa, representing 2.9% of the world market [2].

The *gulupa* fruit selected for export is harvested in an intermediate ripening stage (3 or 4) on the scale proposed by Pinzón et al. [3]. Maximum pulp yields are reached in this state, representing between 35 and 50% of the total weight. *Gulupa* is an excellent source of ascorbic acid, thiamine, riboflavin, niacin, calcium, and phosphorus. Its pulp is used to prepare juices and soft drinks. It has essential nutrients with antioxidant properties, such as vitamins, polyphenolic compounds, carotenoids, anthocyanins (cyanidin-3-O- β -D-glucopyranoside), and amino acids that make it attractive for consumption [4,5].

As it is a climacteric fruit, during the postharvest, transport, storage, and marketing period, it continues its normal ripening process, which can cause it to reach maturity before reaching the final consumer, losing its organoleptic quality and causing economic losses for exporting companies of up to 15% of the total volume shipped [5]. Therefore, the transformation of *gulupa* into the pulp as a minimally processed product represents for producers the opportunity to add value to their product and at the same time reduce exportation over costs by transporting less than half the volume, a process that would allow them to control adequately the quality of the product, preserving its nutritional characteristics and obeying changes in consumer preferences. Emerging technologies for food preservation and transformation seem to be the best alternative to achieve the mentioned characteristics. This evolution forces food industries to adapt to new production techniques and current market demands, integrating social and natural sciences, engineering, and technology [6]. These technologies, including high pressure, electrical pulses, and ultrasound, are specially designed for economy, simplicity, and energy efficiency [7].

Generally, ultrasound (US) is used and referred to as high- and low-energy applications. When the frequency is between 20 and 100 kHz, it is known as high-energy ultrasound (greater than 1 Wcm^{-2}) or low frequency. When the frequency is more significant than 100 kHz, the energy supplied is less than 1 Wcm^{-2} . High doses of energy provided to the sample can have the desired effects with the application of ultrasound, but at the same time, it can generate adverse effects such as the degradation of some compounds of interest. The previous statement is why the best effects must be found with the least amount of energy applied.

Low-power and high-frequency ultrasound (US) are used to analyze, control, and process, ensuring safe and high-quality food. Its technology uses acoustic energy, nonionizing mechanical energy, and is noninvasive and nonpolluting [8]. This innovative nonthermal technique inactivates microorganisms and enzymes related to degradation in fruits, thus allowing the retention of quality nutrients in fruits and their derivatives. The US's biocidal effect has been attributed mainly to physical principles (cavitation, mechanical effects, micromechanical shocks) and chemicals with the formation of free radicals due to the sonochemical reaction [9]. The technique is considered low-energy, environmentally friendly, and reduces chemical and physical risks, with minimal processing time. The technique is considered low-energy, environmentally friendly, reduces chemical and physical risks, and has minimal processing time [10,11].

US technology is useful in enhancing the rate of food processing techniques such as cutting, filtration, freezing, extraction, pickling, and drying [12,13]. The US's mechanical effect facilitates dehydration through the drying process [14]. Moreover, US improves the freezing process by increasing ice crystals formation before the freeze-drying (FD) [15,16]. Besides, US is a food dehydration method itself, avoiding the decomposition of food due to a low heat application [17] based on the moderate temperatures generated [18].

The effect of ultrasound (US) pretreatment on freeze-dried quince slices was reported by Yildiz and Izli [19]. They showed higher preservation of bioactive compounds and improved physical properties compared to the untreated fruit samples.

Rodriguez et al. [20] demonstrated that ultrasound pretreatment significantly increased the dehydration rate and solid gain of Sanhua plum during osmotic dehydration without affecting the fruits. Žlabur et al. [21] found that the US pretreatment of honeyberry fruits increased the vitamin C content regardless of the drying technology used.

During atmospheric FD of apples, carrots, and eggplants, US application demonstrated a decrease in the drying time and improved the FD process. Colucci et al. [22] demonstrated that the US caused no destructive effect on eggplants' antioxidant potential or quality aspects. The US pretreatment of onions before FD increased the flavonoids, quercetin, and phenolic content up to 20%. It improved the antioxidant activity of dried vegetables, only if the US treatment was for short periods, but prolonged sonication harmed the antioxidant activity and antioxidant content compounds [23].

There is evidence of the benefits of ultrasound treatment in the dairy industry, fruits, and processed juices. However, it has been found that it can behave differently in various food matrices, especially concerning the stability of its biomolecules such as ascorbic acid [24]. Therefore, it is necessary to know the low-frequency ultrasound effect on the *gulupa* pulp as neither the thermal processing nor the sonication effects on the quality parameters of the *gulupa* have been reported to date.

2. Materials and Methods

2.1. Vegetal Material

Gulupa fruits (*Passiflora edulis* f. *edulis*) were selected in a state of maturation three [3], from a crop certified for export by the Colombian Institute of Agriculture (ICA), located in the municipality of Pradera (Valle del Cauca, Colombia) at 2430 m.a.s.l. and coordinates 3°25'39.6" N 76°05'54.6" W.

The *gulupa* fruits were selected according to the appearance of quality and physical characteristics (homogeneous color, size, without bruises and wrinkles). The fruits were washed with a mixture of 70% ethyl alcohol and 5% sodium hypochlorite. The fruits were cut in half to obtain the pulp, and the pulp, including the seeds, was extracted with a sterile spoon according to the procedure described by the Colombian technical standard (NTC 5468:2012) [25], and 100 g of pulp was placed in sterile zip-lock polyethylene bags of size 4 × 5.6 inch (10.16 × 14.224 cm) (Ziploc, Sc Johnson, Racine, WI, USA); each of them was considered an experimental unit.

2.2. Treatments

The low-frequency ultrasound treatments consisted of applying an ultrasound power of 325 W (UCD—150 Raypa Leading Lab Technologies, Terrassa, Barcelona, Spain) to the *gulupa* pulp, varying the frequency (30 and 40 kHz) and the exposure time (10, 20, and 30 min), obtaining six (6) treatments and one (1) control sample. The treatments chosen in this work were based on the results from [10,11,24,26], in which frequencies of 20 kHz were used.

During the ultrasound treatment, agitation within the bag was not required, due to the dispersion effect caused by the technique; it induces a longitudinal displacement of particles [8], favored by the low viscosity of the pulp. During the ultrasound treatments, an ice bath was applied to maintain the *gulupa* pulp samples' temperature at 5 °C. After performing the treatments, the samples were stored at 5 °C until their use in the tests. All tests were carried out in triplicate every four days until 20 days were completed.

2.3. Titratable Acidity and pH

Titrate acidity was determined by standard procedure with a 0.1 N sodium hydroxide solution in phenolphthalein's presence as an indicator. The pH of the pulp was determined directly with an electrode probe connected to a potentiometer (model FE20

Mettler Toledo, Columbus, OH, USA) according to the international standard (AOAC 981.12, 1982) [27].

2.4. Soluble Solids (SS)

They were determined in a small pulp juice sample using an Atago refractometer (0–32 ATC Fukaya-shi, Saitama, Japan). The results were expressed in °Brix.

2.5. Trolox Equivalent Antioxidant Capacity (TEAC)

2.5.1. ABTS^{•+} (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Cationic Radical

The ability to trap free radicals was measured by the ABTS cation radical discoloration test, according to [28]. For this, 30 µL of each sample conveniently diluted in phosphate buffer solution pH 7.4 (PBS) was mixed with 2.97 mL of the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) solution prepared and standardized to 0.70 ± 0.02 absorbance units. The discoloration of ABTS^{•+} in the sample's presence was monitored at 734 nm after six minutes of reaction using a Genesys 20 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Antioxidant activity was determined as µmol of Trolox-equivalent per gram of pulp using the ratio of the correlation coefficient of the sample's dose–response curve and the dose–response curve of the standard.

2.5.2. DPPH· (2,2-diphenyl-1-picrylhydrazil) Radical Scavenging Assay

The activity of removing free radicals from the pulp was determined according to [29] with some modifications. The stock solution was prepared by dissolving 2.5 mg of DPPH· (2,2-diphenyl-1-picrylhydrazil) in 100 mL of methanol and adjusting the solution to absorbance 0.70 ± 0.02 at 515 nm. For free radical determinations, 100 µL of diluted pulp was used and mixed with 2.9 mL of standardized DPPH·. The mixture was vortexed (IKA, Staufen im Bresigau, Germany) and kept in the dark for 30 min, and then the absorbance was read on a spectrophotometer. The results were expressed as µmol Trolox-equivalent per gram of pulp. The analyses were carried out in triplicate.

2.6. Determination of Ascorbic Acid

Determination of the ascorbic acid content was carried out using a Thermo scientific ultimate 3000 HPLC liquid chromatography (Waltham, MA, USA), equipped with a UV/VIS detector at 245 nm. The sample mixture preparation consisted of a 20 µL aliquot of pulp diluted in 20 mL of distilled water using a dark bottle to protect it from light. Then, it was filtered through a 0.22 µm PVDF filter, and 20 µL was injected into a C-18 column (2.7 µm, 150 mm × 2.1 mm) at 30 °C using a 5 mM phosphate buffer solution at pH 2.5 as a mobile phase and at a flow rate of 0.2 mL min⁻¹. The peak was identified by comparing the ascorbic acid L-(+) standard's retention time, according to the external standard calibration curve for L-(+) ascorbic acid [30]. The results were expressed in mg/100 g of pulp.

2.7. Color Determination

Colorimetric analysis of the pulp was determined using a CM-700d spectrophotometer (Konica Minolta, Osaka, Japan) with the following settings: Illuminant D65, observer 10°. The pulp was deposited in a black cylinder 3 cm deep. The CIELAB color coordinates (L*, a*, and b*) and ΔE* were determined. The reported values correspond to the average of 5 measurements. The ΔE* parameter was calculated with the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

2.8. Microbiological Analysis

Following the method described by the Colombian technical standard (NTC 5468:2012) [25], 100 µL of each of the dilutions of the treatments (10^{-1} to 10^{-6} in buffered peptone water, 0.1% w/v) was sown in the following culture media: For Enterobacteriaceae in Crystal Violet-Neutral Red-Bile-Glucose Agar (VRBD) for 48 h at 37 °C; for aerobic mesophylls in

Plate Count Agar (PCA) for 24 h at 30 °C; for psychrophiles in PCA at 4 °C for 96 h; for yeasts on Yeast Peptone Dextrose (YPD) at 30 °C for 24 h; and for filamentous fungi on potato dextrose agar (PDA) for 96 h at 25 °C.

2.9. Statistic Analysis

The results were analyzed using one-way analysis of variances (ANOVA) with Minitab 18 statistical software. The significant differences between means ($p < 0.05$) were determined with Dunnett's test. All data are presented as mean values \pm standard deviation. The extraction and treatments were performed in duplicate (one month separate), and the tests were performed in triplicate.

3. Results and Discussions

3.1. Titrable Acidity and pH

The effect of ultrasound on the *gulupa* pulp's pH did not show significant differences ($p > 0.05$) between the treatments during the 20 days of observation (average export and distribution). The values ranged from 2.72 ± 0.01 to 2.86 ± 0.02 (Table 1). Similar results were reported for juices with ultrasound treatment of *Citrus microcarpa* (Lima kasturi) and *Malus domestica* (Apple), where the changes in pH due to ultrasound treatment were meager, from 4.0 to 3.9 and from 2.68 to 2.66, respectively [31,32]. The low pH values in the control and treatments that remain stable over time are mainly due to a self-regulating or buffering system involving citric acid [5,33] like that found in *Passiflora edulis* f. *flavicarpa* [34].

Table 1. Changes in pH during storage at 4 °C of *Passiflora edulis* f. *edulis* Sims (*gulupa*) pulp subjected to low-frequency ultrasound treatments.

Power (kHz)	Time (min)	Days after Treatment					
		0	4	8	12	16	20
Control		2.81 ± 0.02	2.80 ± 0.11	2.80 ± 0.01	2.76 ± 0.01	2.76 ± 0.01	2.77 ± 0.03
	10	2.72 ± 0.01	2.79 ± 0.01	2.75 ± 0.02	2.73 ± 0.01	2.78 ± 0.02	2.74 ± 0.01
30	20	2.77 ± 0.01	2.80 ± 0.03	2.78 ± 0.01	2.76 ± 0.01	2.80 ± 0.01	2.81 ± 0.02
	30	2.86 ± 0.02	2.74 ± 0.01	2.80 ± 0.01	2.77 ± 0.01	2.78 ± 0.01	2.73 ± 0.01
40	10	2.81 ± 0.01	2.76 ± 0.01	2.78 ± 0.02	2.74 ± 0.01	2.79 ± 0.01	2.75 ± 0.02
	20	2.76 ± 0.02	2.75 ± 0.02	2.76 ± 0.01	2.72 ± 0.01	2.77 ± 0.01	2.77 ± 0.01
	30	2.81 ± 0.01	2.74 ± 0.01	2.77 ± 0.01	2.78 ± 0.01	2.80 ± 0.02	2.78 ± 0.01

Data expressed as mean \pm standard deviation of three repetitions. No statistically significant differences were found between the treatments.

In titratable acidity, there was a significant increase ($p < 0.05$) between day 0 and 4 for all treatments and the control (Table 2), due to the effect of obtaining the pulp, where the opening of the fruit begins to release some organic acids present at the level of the membranous sacs. After day four, small acidity changes were apparent, and at the end of the evaluation of the shelf life, the highest concentrations were presented in the control and the 30 min treatments. It is known that the accumulation of organic acids is the result of interactions between metabolism and vacuolar storage affected by environmental factors such as temperature and the opening of the fruit in this case [35]. As for other fruits, many factors affect the production of organic acids during the useful life of the *gulupa* pulp.

No differences were observed between the treatments' power (30 or 40 kHz), only in the exposure time. The 10 and 20 min treatments showed a significant decrease in acidity concerning the control (day 20), attributable to the deactivation of enzymes, which interrupts some metabolic processes involving organic acids, which is why the acidity could remain constant [36], observing a decrease concerning the control treatment. While the effect of ultrasound in treatments for 30 min was longer, it could favor the release of organic acids from the cell cytoplasm to the outside, caused by the rupture of the cell membrane when the enzymatic activity is decreased [37,38]. Therefore, in 30 min treatments, the effect increased acidity, matching the natural maturation effect that was carried out in the control treatment.

Table 2. Titratable acidity (g citric acid/100 g of pulp) during storage at 4 °C of *Passiflora edulis* f. *edulis* Sims (*gulupa*) pulp subjected to low-frequency ultrasound treatments.

Power (kHz)	Time (min)	Days after Treatment					
		0	4	8	12	16	20
Control		4.4 ± 0.2aA	5.5 ± 0.1bC	5.6 ± 0.2bcC	5.5 ± 0.2bB	5.7 ± 0.1cB	5.9 ± 0.1dB
	10	4.3 ± 0.2aA	5.0 ± 0.2bA	5.3 ± 0.3bB	5.4 ± 0.1bcB	5.4 ± 0.1bcA	5.5 ± 0.1cA
30	20	4.3 ± 0.2aA	5.2 ± 0.1bA	5.4 ± 0.4bcBC	5.4 ± 0.3bcB	5.3 ± 0.2bA	5.6 ± 0.1cA
	30	4.2 ± 0.2aA	5.2 ± 0.1bA	5.3 ± 0.2bB	5.3 ± 0.1bA	5.5 ± 0.2bcA	5.8 ± 0.3cB
	10	4.2 ± 0.3aA	5.2 ± 0.5bAB	5.2 ± 0.3bA	5.1 ± 0.4bA	5.3 ± 0.2bcA	5.6 ± 0.3cA
40	20	4.3 ± 0.3aA	5.3 ± 0.8bBC	5.0 ± 0.5bA	5.4 ± 0.1bB	5.4 ± 0.1bA	5.4 ± 0.4bA
	30	4.3 ± 0.2aA	5.2 ± 0.1bA	4.9 ± 0.4bA	5.3 ± 0.2bcA	5.4 ± 0.2cA	5.7 ± 0.1dB

Data expressed as mean ± standard deviation of three repetitions. In the same row, different lowercase letters indicate statistically significant differences between treatment days ($p < 0.05$). In the same column, different capital letters indicate statistically significant differences between the treatments ($p < 0.05$).

3.2. Soluble Solids (SS)

The effect of ultrasound on the total solids of the *gulupa* pulp showed significant differences ($p < 0.05$) in the applied treatments (Table 3). Ultrasound at 30 kHz for 30 min demonstrated an increase (from 13.4 to 14.8% w/v), as well as those at 40 kHz for 20 and 30 min (from 13.4 to 13.9 and 13.7% w/v , respectively). These treatments may provide enough energy to break the high-molecular-weight chains of carbohydrates [39]. There was a decrease in soluble solids when ultrasound was applied at 40 kHz for 10 min (from 13.4 to 12.7% w/v). At the end of the useful life evaluated, a decrease in soluble solids was only observed in the control and not between the treatments, which is consistent with studies carried out with apple juice (*Malus domestica*) [32], Carrot juice (*Daucus carota* L) [40], strawberry juice (*Rubus ulmifolius*) [41], pear juice (*Pyrus communis*) [42], and orange juice [43]. The decrease may be because ultrasound can cause enzyme deactivation and hinder the process of breaking down organic acids into sugars [36].

Table 3. Soluble solids (% w/v) during storage at 4 °C of *Passiflora edulis* f. *edulis* Sims (*gulupa*) pulp subjected to low-frequency ultrasound treatments.

Power (kHz)	Time (min)	Days after Treatment					
		0	4	8	12	16	20
Control		13.4 ± 0.2bB	13.4 ± 0.1bB	13.4 ± 0.1bB	13.4 ± 0.1bB	13.1 ± 0.1aB	13.0 ± 0.1aA
	10	13.6 ± 0.3aB	13.5 ± 0.2aB	13.3 ± 0.1aB	13.3 ± 0.1aB	13.4 ± 0.1aB	13.3 ± 0.1aB
30	20	13.5 ± 0.2aB	13.4 ± 0.1aB	13.3 ± 0.1aB	13.3 ± 0.2aB	13.4 ± 0.1aB	13.4 ± 0.1aB
	30	14.8 ± 0.1aD	14.8 ± 0.1aD	14.7 ± 0.1aD	14.7 ± 0.1aD	14.7 ± 0.1aD	14.7 ± 0.1aD
	10	12.7 ± 0.1aA	12.8 ± 0.1aA	12.9 ± 0.2aA	12.9 ± 0.2aA	12.8 ± 0.1aA	12.8 ± 0.1aA
40	20	13.9 ± 0.1aC	13.8 ± 0.1aC	13.8 ± 0.1aC	13.8 ± 0.0aC	13.9 ± 0.1aC	13.9 ± 0.1aC
	30	13.7 ± 0.1aC	13.7 ± 0.1aC	13.8 ± 0.1aC	13.7 ± 0.1aC	13.7 ± 0.1aC	13.7 ± 0.1aC

Data expressed as mean ± standard deviation of three repetitions. In the same row, different lowercase letters indicate statistically significant differences between treatment days ($p < 0.05$). In the same column, different capital letters indicate statistically significant differences between the treatments ($p < 0.05$).

3.3. Trolox-Equivalent Antioxidant Capacity (TEAC)

In *gulupa* pulp, the values found in TEAC were 1.13 ± 0.01 $\mu\text{mol TE/g}$ measured as ABTS, and 3.3 ± 0.1 $\mu\text{mol TE/g}$ measured as DPPH (Figure 1). These results are close to those reported for *gulupa* with a state of more advanced maturation (ABTS 3.93 to 4.10 $\mu\text{mol TE/g}$) [44]. In general, it was found that sonicated *gulupa* pulp has a great antioxidant capacity, like other *Passifloras* such as *P. ligularis* Juss (granadilla) and *P. foetida* (wild passion fruit) [45,46].

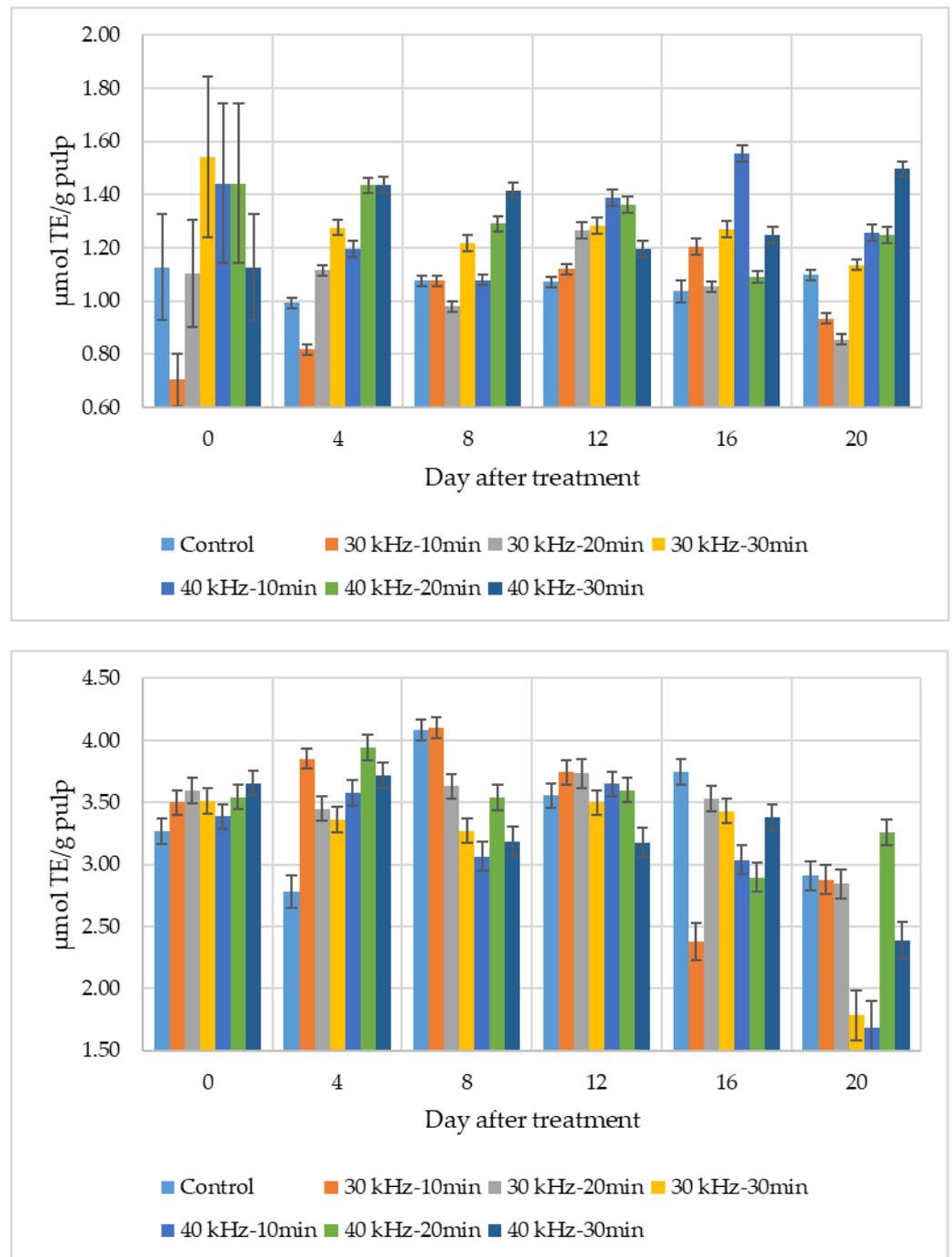


Figure 1. Changes in Trolox-Equivalent Antioxidant Capacity (TEAC) measured as ABTS•⁺ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Cationic Radical Assay (**Top**) and DPPH• (2,2-diphenyl-1-picrylhydrazil) Radical Scavenging Assay (**Bottom**) during storage at 4 °C in *Passiflora edulis* f. *edulis* Sims pulp (*gulupa*) subjected to low-frequency ultrasound treatments. Data expressed as mean ± standard deviation of three repetitions.

It was observed that ultrasound treatments showed a tendency to increase TEAC significantly (day 0) from 1.13 ± 0.01 to 1.54 ± 0.01 (ABTS) and from 3.3 ± 0.1 to 3.7 ± 0.1 (DPPH); only 30 kHz 10 min treatment (measured as ABTS) presented a significant decrease in the control. In general, the increase in antioxidant capacity is possibly due to a more significant disruption of cell walls, facilitating the release of phenolic residues attached to traces of pectin, cellulose, hemicellulose, and lignin in the cell wall [47]. Similar results were reported in *Fragaria × ananassa* (strawberry) stored at 4 °C for 15 days, and *Actinidia*

delicious (Kiwi) juice sonified with the US of 33 kHz for 10 min and 20 kHz for 16 min, respectively [26]. In both investigations, a positive effect of this property was noted on the sonicated samples. This increase in antioxidant capacity with ultrasound treatment can also be attributed to the hydroxylation of flavonoids or the generation of hydroxyl radicals that increase the hydroxylation of food materials [36].

Storage did not show a marked tendency to increase or decrease the antioxidant activity significantly. There were highs and lows in each of the treatments; this property of the sonicated juice could be directly attributed to the US's cavitation, which could have caused the changes observed over the days [31].

It should be noted that ultrasound treatments do not tend to decrease antioxidant activity. Results are opposite to those identified in *Passiflora caerulea*, where it was found that in fresh and pasteurized juice, this property decreased from day 4 of storage [48]; additionally, refrigerated fresh fruit juices (for 29 days) have been reported to lose antioxidant potential [49].

According to the experimental conditions, it is considered that around 16 days of treatment is the most recommended time for the consumption of antioxidants, in agreement with Franco et al. 2014 [50]. On day 20, there was a decrease in TEAC measured as DPPH, possibly due to the decrease in some low-polarity antioxidant molecules, which react to a greater extent with this reagent.

3.4. Ascorbic Acid Content

An initial content of 42.7 ± 0.2 mg ascorbic acid/100 g of pulp was found, similar to those reported by Franco et al. [44] and Pertuzatti et al. [30] in organic crops in Brazil (Table 4).

Table 4. Ascorbic acid content (mg/100 g of pulp) during storage at 4 °C of the pulp of *Passiflora edulis* f. *edulis* Sims (*gulupa*) subjected to low-frequency ultrasound treatments.

Power (kHz)	Time (min)	Days after Treatment					
		0	4	8	12	16	20
Control		42.7 ± 0.2cB	42.4 ± 0.1cA	40.9 ± 2.6bA	40.7 ± 0.1bB	42.9 ± 8.5cD	21.6 ± 2.9aA
	10	39.4 ± 1.1bA	49.7 ± 4.9dC	48.5 ± 1.5dC	43.8 ± 4.4cB	42.0 ± 0.2cD	22.1 ± 3.0aA
30	20	40.1 ± 2.3cA	45.7 ± 3.7dB	45.8 ± 3.8dB	36.7 ± 3.0bA	34.5 ± 2.3bA	20.9 ± 2.7aA
	30	42.9 ± 0.7cB	46.6 ± 4.7dB	48.7 ± 2.0dC	41.0 ± 2.1bB	38.7 ± 2.4bC	23.0 ± 2.6aB
40	10	41.7 ± 1.2cB	43.4 ± 3.4dA	44.2 ± 2.8dB	33.9 ± 3.2bA	31.8 ± 2.4bA	24.5 ± 2.1aB
	20	42.0 ± 1.7cB	46.9 ± 5.8dB	49.1 ± 3.1dC	40.3 ± 1.5bB	38.3 ± 3.8bC	24.1 ± 2.8aB
	30	43.1 ± 1.7cC	46.3 ± 4.2dB	48.3 ± 4.1dC	40.0 ± 4.7bB	36.6 ± 1.6bB	23.8 ± 3.5aB

Data expressed as mean ± standard deviation of three repetitions. In the same row, different lowercase letters indicate statistically significant differences between treatment days ($p < 0.05$). In the same column, different capital letters indicate statistically significant differences between the treatments ($p < 0.05$).

A significant decrease ($p < 0.05$) in the content of ascorbic acid was observed in the treatments at 30 kHz for 10 and 20 min on day zero (Table 4). A small production of reactive species due to the lower degassing rate than the other treatments allows reactive species formation. In this way, aerobic oxidation could explain the ascorbic acid losses reflected in these two treatments, which agrees with the trend found in several juices and fresh fruit pulps processed by ultrasound [51], as the juice of *Physalis peruviana* L. (Uchuva) [39] in *Passiflora edulis* f. *flavicarpa* (yellow passion fruit) [52]. Ascorbic acid's oxidation can be caused by the addition of oxygen during the opening of the fruit and handling when working the pulp and by the formation of free radicals during the application of ultrasound, which can form reactive species such as H, OH, HO₂, O, and H₂O₂, and therefore cause its oxidation immediately [51].

On the contrary, there was a significant increase in ascorbic acid content in the treatments, reaching values of up to 49.1 ± 3.1 mg ascorbic acid/100 g of pulp on day 8 of storage. The increase might be caused by the fruit's opening that begins to slowly release some organic acids present at the membranous sacs' level as a protection mechanism,

including ascorbic acid. From day 12, the content began to decrease, presenting the lowest values on day 20 of storage. In general, the 30 kHz treatments for 30 min and all the 40 kHz treatments showed the highest ascorbic acid values at the end of the storage period, which suggests that degassing is essential to prevent the oxidation of the pulp components.

3.5. Color Measurements

Color is one of the essential appearance attributes in the pulp market, as it is the first quality parameter evaluated by consumers and is critical in the acceptability of the product [53].

The *gulupa* pulp is not uniform in its appearance and presents two color patterns, a dark color contributed by the seed and its yellow color of the aril. Due to this, the parameters presented significant variability throughout the experiment. Table 5 shows the analysis of the pulp L*, a*, and b* coordinates. A significant increase in the L*, a*, and b* parameters was found with application of ultrasound treatments. The increase in luminosity (L*) may result from the decrease in particle size that increases light reflection and increases its luminosity [54] and carotenoid destruction. Similar results were reported by Gómez-López [52] in the juice of *Passiflora edulis* f. *flavicarpa* (yellow passion fruit). This increase in L* value can also be attributed to the partial precipitation of unstable particles in the juice, as observed in orange juices subjected to pasteurization processes [55] and orange juice treated by high-intensity pulsed electric fields caused by the increase in temperature [56], which also occurs during prolonged storage as observed in the control. In US treatments, the rise in temperature at the microsites may cause the precipitation of unstable compounds.

Table 5. Color analysis of *Passiflora edulis* f. *edulis* Sims (gulupa) pulp subjected to low-frequency ultrasound treatments stored at 4 °C.

Power (kHz)	Time (min)	Parameter Color	Days after Treatment					
			0	4	8	12	16	20
30	Control	L*	41 ± 0aA	40 ± 2aA	40 ± 2aA	42 ± 1aB	42 ± 2aA	47 ± 1bC
	10		49 ± 1dC	42 ± 1bB	42 ± 1bB	39 ± 1aA	44 ± 2cB	42 ± 2bA
	20		42 ± 1aA	42 ± 2aB	42 ± 0aB	44 ± 1bC	46 ± 2cC	46 ± 1cBC
	30		46 ± 2cB	39 ± 1aA	39 ± 1aA	41 ± 1bB	42 ± 1bA	45 ± 1cB
	10		46 ± 3aB	46 ± 2aC	46 ± 2aC	46 ± 1aD	48 ± 2aD	53 ± 3bD
	20		45 ± 3cB	40 ± 1aA	42 ± 1bB	46 ± 1cD	46 ± 1cC	50 ± 0dD
40	30	a*	41 ± 1aA	42 ± 2aB	43 ± 2abB	44 ± 2bC	44 ± 2bB	45 ± 3bB
	Control		6 ± 1aA	8 ± 1cA	7 ± 0bA	5 ± 1aA	5 ± 1aA	8 ± 1cC
	10		11 ± 1cC	9 ± 1bB	8 ± 1bB	7 ± 1aB	6 ± 1aA	6 ± 0aB
	20		7 ± 1aA	8 ± 1bA	9 ± 1bB	9 ± 1bC	6 ± 1aA	7 ± 1aB
	30		8 ± 1bB	8 ± 0bA	8 ± 1bB	7 ± 1aB	6 ± 1aA	7 ± 1aB
	10		11 ± 2bC	10 ± 2bB	10 ± 1bC	8 ± 2aB	6.9 ± 0.3aB	10 ± 1bD
30	20	b*	9 ± 2bB	9 ± 0bB	9 ± 1bB	7 ± 3abB	7 ± 1abB	8 ± 1bC
	30		7 ± 1bA	7 ± 1bA	7 ± 1bA	7 ± 0bB	6 ± 1aA	5 ± 1aA
	Control		20 ± 1aA	23 ± 2bB	23 ± 3abA	27 ± 1cB	27 ± 3cC	28 ± 1dB
	10		32 ± 2bD	23 ± 1aB	23 ± 1aA	24 ± 1aA	23 ± 3aAB	23 ± 1aA
	20		24 ± 1bB	22 ± 1aB	24 ± 2abA	31 ± 3cC	25 ± 1bC	26 ± 3bB
	30		28 ± 2cC	21 ± 0aA	23 ± 2bA	26 ± 1cB	21 ± 1aA	27 ± 2cB
40	10	ΔE*	30 ± 2dC	27 ± 2bC	28 ± 2bB	30 ± 2dC	30 ± 2dD	33 ± 2eD
	20		28 ± 3bC	23 ± 1aB	24 ± 1aA	32 ± 3bC	23 ± 1aB	30 ± 1bC
	30		23 ± 3bB	20 ± 1aA	22 ± 1bA	23 ± 1bA	23 ± 2bB	22 ± 1bA
	Control		0	5 ± 2aB	5 ± 2aA	8 ± 1bB	8 ± 1bC	11 ± 1cB
	10		15 ± 2cC	5 ± 1bB	5 ± 1bA	5 ± 1bA	5 ± 3abB	3 ± 1aA
	20		5 ± 2aA	4 ± 1aB	7 ± 1bB	12 ± 3cC	8 ± 3bC	8 ± 3bB
30	30	ΔE*	10 ± 3dB	3 ± 1abA	4 ± 1bA	7 ± 1cB	2 ± 1aA	9 ± 2dB
	10		13 ± 5bBC	10 ± 3abC	11 ± 3bC	12 ± 3bC	11 ± 1bD	19 ± 4cD
	20		10 ± 4bB	5 ± 1aB	7 ± 2bB	14 ± 3cC	4 ± 1aB	14 ± 1cC
	30		4 ± 3abA	2 ± 1aA	4 ± 1bA	5 ± 1bA	5 ± 2bB	5 ± 2bA

Data expressed as mean ± standard deviation of three repetitions. In the same row, different lowercase letters indicate statistically significant differences between treatment days ($p < 0.05$). In the same column, different capital letters indicate statistically significant differences between the treatments ($p < 0.05$).

The increase in redness (a^*) and yellowness (b^*) may be related to the isomerization of carotenoids present in the *gulupa* pulp [57], which can form compounds with brown color characteristics, similar to those that occur in the browning process. According to Fonteles et al. [11], any change in the values of the parameters a^* and b^* is associated with a simultaneous change in luminosity value (L^*) as observed in this experiment, mainly in shelf life. In addition, they increase with the application of ultrasound treatments but decrease with exposure time (day 0).

The values found for ΔE^* (Table 5) showed that all treatments (day 0) had significant changes compared to the control and that these changes can be perceptible by the human eye ($\Delta E > 2$) [58]. The smallest global color difference (ΔE) for the control was found in the 40 kHz treatment at 30 min through the entire shelf life (day 0 to 20), besides presenting minor variations, possibly related to the lower loss of ascorbic acid in this treatment caused by the rapid elimination of gases dissolved in the pulp.

Most of the treatments with ultrasound showed a ΔE^* decreasing trend, presenting values lower than the control, especially on day 16. The trend continued until day 20 except for samples treated at 40 kHz at 10 and 20 min, which remained at values higher than the control. In the untreated pulp (control), there is a tendency to increase the color parameters L^* , a^* , and b^* , which involves many aspects that can influence the ΔE^* , as was mentioned already. However, these changes can sometimes be desirable because they reinforce its natural color and increase pigment content. In general, sonication treatments can help preserve these pigments [59].

3.6. Microbiological Analysis

In the *gulupa* pulp, no enterobacteria, aerobic mesophilic bacteria, or psychrophilic bacteria were found, neither in the control nor in the samples treated with ultrasound. *Gulupa's* low pH (pH 2.81) may act as a retardant or growth inhibitor of pathogenic bacteria, which generally require substrates with pH values higher than 4.6. Acidic foods such as citrus fruits and their products are considered safe for direct consumption because the survival of microorganisms that generally attack food and are pathogenic for humans hardly grow at low pH levels [31]. Additionally, the growth and development of microorganisms such as bacteria require media rich in protein and low in sugar, contrary to those found in *gulupa* pulp, whose protein content ranges between 0.7 and 0.9% in the different stages of maturation [5], which may mean that the *gulupa* is a problematic substrate for the development of bacteria.

A significant decrease in yeast content was observed due to the effect of ultrasound for the 30 kHz treatments at 20 and 30 min (1.2–1.3 log CFU/mL) and all 40 kHz treatments (1.5–2.1 log CFU/mL) concerning the control (Table 6). This fact is attributed to the ultrasound treatment, where the production of cavitation bubbles in the fluid causes a higher temperature and pressure in the cavitation region, thus achieving the inactivation of the yeasts [32].

Table 6. Yeast count (log CFU/mL) during storage at 4 °C of *Passiflora edulis* f. *edulis* Sims (*gulupa*) pulp subjected to low-frequency ultrasound treatments.

Power (kHz)	Time (min)	Day after Treatment					
		0	4	8	12	16	20
30	Control	4.2 ± 0.2cD	3.5 ± 0.2bC	3.2 ± 0.2bD	2.9 ± 0.2aC	2.8 ± 0.2a	2.8 ± 0.2a
	10	4.2 ± 0.2dD	2.8 ± 0.2cB	2.6 ± 0.2bC	2.5 ± 0.2bB	2.0 ± 0.2a	n.d
	20	3.0 ± 0.2cC	2.7 ± 0.2bB	2.7 ± 0.2bC	2.4 ± 0.2aAB	n.d	n.d
	30	2.9 ± 0.2cC	2.6 ± 0.2bAB	2.5 ± 0.2abC	2.3 ± 0.2aA	n.d	n.d
	10	2.7 ± 0.2cB	2.5 ± 0.1bA	2.3 ± 0.2aB	2.2 ± 0.2aA	n.d	n.d
40	20	2.5 ± 0.3cB	2.4 ± 0.2bA	2.0 ± 0.2aA	n.d	n.d	n.d
	30	2.1 ± 0.2A	n.d	n.d	n.d	n.d	n.d

Data expressed as mean ± standard deviation of three repetitions. In the same row, different lowercase letters indicate statistically significant differences between treatment days ($p < 0.05$). In the same column, different capital letters indicate statistically significant differences between the treatments ($p < 0.05$). Legend n.d not detected.

Ultrasound is considered to act on microorganisms' hydrophobic surfaces, which help collision cavitation bubbles and cause severe damage to the cell wall and, consequently, microbial inactivation [60]. However, some authors state that yeast cells are relatively rigid and are not easy to break, due to the cavitation effect, so the inactivation can also be attributed in large part to the formation of free radicals such as hydrogen peroxide and the release of intracellular proteins [61]. Additionally, the initial inactivation could also be favored by the low pH of the *gulupa* [9], e.g., bacteria, as indicated by Khandpur and Gogate [62]. Microbe inactivation by ultrasound is sufficient when used combined with other decontamination techniques such as extremes of pH. As occurred in this study, a low pH of *gulupa* pulp may increase the antimicrobial efficiency of sonication, possibly attributed to the improved hydroxyl radical production. The results found were higher than those reported by Fan et al. [63] in fresh-cut cucumber, where it was possible to reduce the yeast load by 0.41–0.84 log CFU/g with treatments of 20 kHz for 5 and 15 min. It was also observed that the reduction in yeast colonies increased with the potency and exposure time. According to Adekunle et al. [64] and Bevilacqua et al. [65], potency (as amplitude level) and treatment time are significant in reducing yeast. The lethal effect of ultrasound treatments depends on the type of microorganism due to differences in the composition of their membranes, cell walls, or organelles. [9]. Parameters such as the medium's viscosity, initial microbial load, and processing affect the treatment's susceptibility [59,66]. Generally, sonication cavitation is more effective for Gram-negative bacteria and small round cells than for fungi due to the cell surface-to-volume ratio [59].

The shelf-life study results also established that the yeast count through the 20 days of storage at 4 °C decreased. This decrease in viable yeasts also affected the control treatment, indicating that refrigeration at 4 °C, seen as thermal treatment, helped decrease the yeast load by 2.2 log CFU/mL in the 20 days. The further decrease could be because yeasts remain in an acidic environment in the presence of the generated hydroxyl radicals and H₂O₂ produced during cavitation, directly attacking yeasts cells. However, the lower yeast load was presented initially because of the ultrasound treatments, which favors the thermal inactivation process. For example, in the treatment with 40 kHz for 30 min, the initial load decreased from 4.2 to 2.1 log CFU/mL. Thermal inactivation from a lower load was achieved in only four days, while in the control treatment, it decreased (Table 6).

3.7. Additional remarks

The use of polypropylene bags for packing the pulp and subsequent ultrasound treatment has advantages because it avoids the subsequent contamination in the packing process. However, ultrasound likely affects the package, causing the release of incorporated or unbound substances. It has been demonstrated that ultrasound, through different mechanisms, including cavitation, can degrade polymers by the reducing molecular weight by breaking the most susceptible chemical bond without changing the chemical nature of the polymer [67,68]. Although it occurs in extreme conditions and has been tested only using solvents that facilitate the components' mobility, this degradation can generate a migration to the food [69].

The extension of the technique to more technical industrial processes should evaluate the available ultrasound equipment. Currently, ultrasound applications are based on three methods: Direct application to the product, coupling with the device, and submergence in an ultrasonic bath [8]. The first two techniques offer greater control of the energy delivered to the sample, and they can be used as long as the microbiological safety conditions at the packaging stage allow it. The use of the ultrasound bath is conditioned to migration processes of the packaging toward the food; although there are few studies in this regard, it must be ensured that the migration is below the limits established for implementation. A general aspect in the process is that it is necessary to avoid heating the sample due to the degradation that biomolecules can undergo, which we want to conserve and potentiate; in this sense, refrigeration systems' coupling must be essential in scaling the process.

4. Conclusions

The present study showed that ultrasound treatments increase the TEAC values and ascorbic acid content in the *gulupa* pulp. In the same way, it was detected that the treatments did not affect other physicochemical properties (pH, soluble solids, titratable acidity). The microbiological analysis determined that the ultrasound treatments induced a significant decrease in the yeast content during the shelf life, especially the treatment with greater frequency and longer exposure time (40 kHz, 30 min). The previous finding could become a new way to improve species' safety and quality with similar physicochemical characteristics as it offers a new strategy to enhance and extend the shelf life of chilled *gulupa* pulp.

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