Effects of high-intensity ultrasonic bath on the quality of strawberry juice

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ABSTRACT
The effects of ultrasound (US) application conditions on microbiological, physicochemical, microscopic, and sensory features of strawberry juice were evaluated. Conditions comprised US (40 kHz) for 5, 10, or 15 min at 25, 40, or 50°C. All US treatments reduced the microbial count in juice. US treatment (40°C/10 min; 50°C/15 min) reduced the °Brix value. All treatments maintained the pH, total titratable acidity, phenolic compounds, and antioxidant capacity of the samples. The thermal treatment sample presented a higher decrease in bioactive compounds. Hue angle values and changes in optical microscopy have increased as treatment temperature and time increased. The sample sonicated at 50°C for 15 min presented lower sensory acceptance indices for all the evaluated attributes. Ultrasound application at 50°C for 5 min presented effective juice properties’ conservation, a good sensory acceptance, and is a promising alternative to help preserve strawberries.

Efectos del baño de ultrasonido de alta intensidad en la calidad del jugo [zumo] de fresa
En el presente estudio se evaluaron los efectos de las condiciones de aplicación de ultrasonidos (US) en las características microbiológicas, fisicoquímicas, microscópicas y sensoriales del jugo de fresa. Las condiciones comprendieron la aplicación de ultrasonido (40 kHz) durante 5, 10 o 15 minutos a 25, 40 o 50°C. Se pudo constatar que todos los tratamientos de US redujeron el recuento microbiano en el jugo. Además, se observó que el tratamiento de US (40°C/10 min; 50°C/15 min) redujo el valor °Brix. Por otra parte, se constató que todos los tratamientos aplicados mantuvieron el pH, la acidez total, los compuestos fenólicos y la capacidad antioxidante de las muestras. La muestra sometida a tratamiento térmico mostró una mayor disminución de los compuestos bioactivos. Los valores del ángulo de tonalidad y los cambios en la microscopía óptica aumentaron a medida que se incrementaban la temperatura y el tiempo de tratamiento. La muestra sometida a prueba sónica a 50°C durante 15 minutos registró menores índices de aceptación sensorial para todos los atributos evaluados. La aplicación de ultrasonido a 50°C durante 5 minutos dio lugar a una efectiva conservación de las propiedades del jugo, una buena aceptación sensorial, por lo que es una alternativa prometedora para ayudar a conservar la fresa.

1. Introduction
Juice consumption has increased worldwide due to its taste and nutritional benefits (Priyadarshini & Priyadarshini, 2018). The juice market is an upward trend due to consumers’ choice for healthier, natural drinks, as well as to the diversification of products with better aroma, flavor and color (Priyadarshini & Priyadarshini, 2018; Yılmaz et al., 2019; Wang et al., 2019a). The microbiological quality of juices is affected by several factors; product contamination can take place at different production stages, from planting to processing ( Ağçam et al., 2018). The food industry uses heat treatments as pasteurization method in order to inactivate enzymes and to reduce the number of microorganisms in food (Swamy et al., 2018). However, heat can damage the physical-chemical, sensory and nutritional features of different products (Tomadoni et al., 2017). Thus, there is interest in developing alternative and promising technologies that do not cause undesirable losses and help preserve products’ features (Yi et al., 2017).

Therefore, ultrasound use has been investigated as an alternative technology to conventional heat treatments, since it enables flavor preservation and higher energy savings (Tomadoni et al., 2017; Yılmaz et al., 2019). Expansion and compression zones are alternately formed during acoustical cavitation processes generated by high powers; small bubbles tend to grow during the expansion cycle due to local pressure lower than the liquid’s vapor pressure (Bermudez-Aguirre, 2017; Kentish, 2017). The surface area of the bubble reduces during the compression stage. Bubble implosion happens at different pressure zones, and it generates high temperature (up to 5000°C) and pressure (up to 1000 atm) regions (Kentish, 2017; Mukhopadhayya & Ramaswamy, 2012). Free radicals are also formed during treatment; they have oxidative power and damage microorganisms’ DNA, whose extension becomes ruptured and fragmented (São José et al., 2014). Ultrasound is a green, safe and non-toxic technology. Different types of ultrasound...
can be employed for juice conservation, generally using two types of equipment: an ultrasonic probe (contact-type) or an ultrasonic bath (noncontact-type) (Rojas et al., 2020, 2017). The first one consists of a single transducer connected to a sonic horn, usually made from a titanium alloy. This type of ultrasound is more powerful than an ultrasonic bath-type, and for this reason, they are frequently applied to treat liquid food placed directly in a recipient (Rojas et al., 2020). However, contact-type systems can pose some hazards to consumers because during the sonication process pitting of ultrasonic probes by intense cavitation phenomena may occur, that consequently results in the erosion of the radiating surfaces, and contamination of food products treated in direct contact with the probes (Kentsish & Feng, 2014). An alternative to this challenge is to use a noncontact-type system such as the ultrasonic bath equipment. An ultrasonic bath consists of a water bath that presents one or more transducers usually at the bottom of the bath (Rojas et al., 2020). This equipment propagates acoustic waves through a liquid. In fact, food can be treated directly by placing liquid food in the bath or indirectly by placing the food in containers and then into the ultrasonic bath with water (Rojas et al., 2020).

Ultrasound equipment can be applied alone or in association with other technologies such as pressure (manosonication), heat (thermosonication) or pressure and heat (manothermosonication) (Chandrapala et al., 2012). Moreover, ultrasound wave application to liquid food, such as juices, can have desirable physical and mechanical effects on samples and cause microscopic changes in plant tissues (Rojas et al., 2016). There is a global interest in developing new research into innovative non-thermal processing technologies that could be an alternative to conventional thermal processes in food, including fruit juices (Swamy et al., 2018). The use of ultrasound alone is not ideal to eliminate microorganisms and requires a long time of processing (C. X. Cheng et al., 2020). Bhat and Goh (2017) performed analysis on strawberry juice treated with ultrasound (25 kHz, power 70% at 20°C for 15, and 30 min). Although the treatment conditions proposed by the authors have maintained the physicochemical quality and improved cloudiness, turbidity, and bioactive compounds, no reduction in microbial load was observed even after 30 min of treatment. Similarly, Tomadoni et al. (2017) found no reduction in microorganisms or any loss of quality attributes after strawberry juice ultrasound-bath processing at 20°C, 40 kHz for 10 and 30 min. Wang et al. (2019b) used probe-type ultrasound equipment at 20 kHz, without association with temperature, for 4, 8, 12 and 16 min to study the effect on bioactive compounds, color, and microstructure of strawberry juice. The authors showed that the increase in time processing changed the microstructure and improved the bioactive compounds.

It is important to analyze and compare the effect of different times and temperatures on the overall quality parameters of juice, including the microscopic structures and sensory attributes, and not just the isolated effect on a specific parameter, aiming to understand the impact of ultrasound on these characteristics. Therefore, and based on what was described above, the current study aimed to investigate the effects of different ultrasound-bath-type application conditions on microbiological, physicochemical, microscopic, and sensory features of strawberry juice. At the same time, a thermal treatment was also applied because it is considered the conventional process employed for fruit juice conservation.

2. Materials and methods

2.1. Experimental design, juice preparation and treatments

The experiment followed a completely randomized design, and it was conducted in triplicate for each treatment. Strawberries (Fragaria x ananassa Duch.) were purchased in the local market in Vitória City (Espírito Santo State, Brazil) and transported in isothermal boxes. Fruits with good appearance were washed in running water to remove dirt and then, sanitized with 100 mg/L sodium hypochlorite (Hidrosteril®, Itapevi, São Paulo, Brazil) for 15 min. The water content used to prepare the juice corresponded to 50% of strawberry weight (2:1 of strawberry and water, respectively). Strawberry juice was prepared in a previously sanitized domestic blender (Arno Clic’Lav Top LN72, Arno®, Brazil). Next, 50 mL was distributed in sterile tubes and subjected to thermal treatment in bath (90°C for 60 seconds) or ultrasound treatments (25, 40 or 50°C for 5, 10 and 15 min). The ultrasound-treated sample was placed in the center of the equipment at 15 and 7.5 cm from the sides. The ultrasound equipment used in the experiment was a bath type, at a frequency of 40 kHz (Branson®, Model CPX3800H, 110 W – 40 kHz, Danbury, United States). Samples that did not undergo any conservation treatment were used as control. After treatments, samples were immediately cooled and subjected to analyses.

2.2. Microbiological analyses

The current study has investigated natural juice contamination with aerobic mesophilic bacteria, yeasts and molds, and coliforms at 35°C, as well as with Escherichia coli, based on the Compendium of Methods for The Microbiological Examination of Foods (2001). Pour plate technique application in standard agar was used to count aerobic mesophilic bacteria (Himedia®); plates were incubated at 35°C for 48 h. Yeasts and molds were incubated at 25°C for 5 to 7 days after spread plate technique application in potato dextrose agar (Fluka Analytical®). Petrifilm plates (3 M®, Maplewood, United States) were used to analyze coliforms at 35°C and Escherichia coli, according to manufacturer’s recommendation. Blue colonies with gas represented E. coli, whereas red and blue colonies with gas represented coliforms at 35°C. Plating was conducted in duplicate (two dilutions); results were expressed as colony forming units per mL (CFU/mL).

2.3. pH, total soluble solids, and total titratable acidity

Sample pH was measured with the aid of a pHMeter (Tecnopon®, Piracicaba, São Paulo, Brazil). Analogical refractometer (Instrutherm®, Freguesia do Ó, São Paulo, Brazil) was used to measure the total soluble solids (°Brix) content by using three drops of juice per refractometry at 25°C; results were expressed as °Brix. Total titratable acidity was determined through titration under stirring with 0.1 N NaOH solution. Analyses were conducted based on official methods (AOAC, 2012).
2.4. Ascorbic acid, anthocyanins, total phenolic compounds, and antioxidant capacity

Ascorbic acid content was estimated through titration using 10 mL of juice; results were expressed as mg/100 mL official methods (AOAC, 2012). Anthocyanins were analyzed based on Lees and Francis (1972). Sample absorbance was measured with a spectrophotometer (Visor Spectrum®, UV-Visible light SP-2100UV) at 520 nm; results were expressed as mg/100 mL of juice. An extraction procedure aimed at estimating total phenolic compounds and antioxidant capacity was performed based on Bloor (2001). Folin-Ciocalteu reagent (Sigma-Aldrich®) was used to estimate total phenolic compounds based on the protocol described by Singleton et al. (1999). Absorbance was measured in spectrophotometer (Visor Spectrum®, UV-Visible light SP-2100UV) at 765 nm. Analytical curve was generated at different gallic acid concentrations (Y = 17.89X + 0.050; R² = 0.995) to enable estimating phenolic compounds; the results were expressed as mg of gallic acid equivalents/100 mL. Antioxidant capacity was determined based on the 1.1-diphenyl-2-picrylhydrazyl (DPPH •, Sigma-Aldrich®) test, according to the method described by Blois (1958). Sample absorbance was measured in a spectrophotometer (Visor Spectrum®, UV-Visible light SP-2100UV) at 517 nm; antioxidant capacity was calculated and expressed as percentage (%).

2.5. Color determination

Instrumental color was determined in color spectrophotometer used to measure reflectance and transmittance (Hunter Associates Laboratory®, model XE, Reston, Virginia, United States). The device was set at illuminating condition D65 (average daylight) and 10° field of view (standard observer). Samples were placed on plates in order to have their surface color measured in at least four replicates. The color parameters measured in the current study were brightness (L*), redness (a*) and yellowness (b*); L*, a* and b* values were converted into hue angle (H°), chroma (C°), browning index (βI) and total color difference (∆E) (Pathare et al., 2012).

2.6. Optical microscopy

Microscopic features of the juice were evaluated based on Rojas et al. (2016). Juice samples (~20 µl) were dispersed on a glass slide and observed at 10x magnification in an optical microscope (Carl Zeiss® Microscopy, model 37081, Göttingen, Germany) equipped with a digital camera (Carl Zeiss® AxioCam ERC 5s, Germany). Images of each sample were captured at least five times and subjected to qualitative analysis.

2.7. Sensory acceptance analysis

The study was approved by the Ethics and Research Committee of the Health Sciences Center at the Federal University of Espírito Santo (Protocol number 56249616.1.0000.5060). All the participants (n = 100) were volunteers and signed the Informed Consent Form. The most efficient treatments for inactivating microorganisms were determined through sensory analysis. Sensory acceptance tests were performed in individual compartments, under white light, based on Minim (2010). Color, aroma, appearance, consistency, flavor and overall acceptance were the evaluated attributes. The hedonic scale used to score the attributes ranged from “1 = I really dislike it” to “9 = I really like it”. In addition, the acceptance rate of each sample was calculated based on Teixeira et al. (1987) and expressed as the Acceptance rate (%). The juice was prepared 1 day before the analysis and stored at 7 ± 1°C. The samples (50 ml) were offered to participants at cooling temperature, in monadic form and coded with three random digits in random order. A glass of water at room temperature was also offered to participants, so they could rinse their mouth and clean their palate before each evaluation.

2.8. Statistical analyses

Data were subjected to analysis of variance (ANOVA) and means were compared through Tukey test in the SAS® online software, at a significance level of 0.05.

3. Results and discussion

3.1. Effect of treatments on natural microbiological contamination of strawberry juice

None of the samples has shown natural contamination with E. coli. All ultrasound treatments have significantly reduced (p ≤ 0.05) the number of aerobic mesophilic bacteria, molds and yeasts, as well as of coliforms at 35°C, in comparison to the control (Table 1). The evaluated microbial groups presented progressively significant decrease (p ≤ 0.05) as the ultrasound treatment temperature increased. Ultrasound application time increase did not significantly increase microbial reductions when the same temperature was applied. Ultrasound treatments at 25°C have significantly decreased the number of aerobic mesophilic bacteria, yeasts and molds in the evaluated samples; however, they did not reduce the number of coliforms at 35°C (p ≤ 0.05) in them. The most intense ultrasound treatment (50°C/15 min) has reduced the number of aerobic mesophilic bacteria, yeasts and molds, and coliforms at 35°C at 2.92, 2.05 and 2.86 log CFU/mL, respectively.

Cavitation is the main mechanism involved in the inactivation of microorganisms by ultrasound. The process consists in formation, growth, and collapse of microbubbles that create intense pressure and heat. Furthermore, other phenomena occur during the application of ultrasound such as agitation, pressure, shock waves, shear forces, micro jets, compression, refraction, acoustic transmission, and formation of free radicals (Bermudez-Aguirre, 2017; Ojha et al., 2018). Bhat and Goh (2017) did not observe a significant reduction in the total number of yeasts and mold counts of fresh strawberry juice subjected to ultrasound treatment at 25 kHz for 15 or 30 min at 20°C. Cassani et al. (2020) treated prebiotic-rich strawberry juice with ultrasound (40 kHz, 20°C, 15 and 30 min), and also did not obtain an immediate effect on reducing the natural contamination of total aerobic mesophilic bacteria, psychrophilic bacteria, and yeast and molds in comparison to controls. However, Yılmaz (2020) applied ultrasound (26 kHz) on red and yellow watermelon juices without temperature association at different times of processing (4, 8, 12 and 16 min), and obtained a satisfactory reduction of total Enterobacteriaceae count, total aerobic plate count, and yeast and mold count.
Similar to results found in the present study, the temperature increase in ultrasound treatments has shown a strong microbial inactivation effect on carrot juice (Pokhrel et al., 2017). Thermosonication technology, which combines ultrasound treatment with moderate heat (37°C to 75°C), is a potential processing alternative to increase microorganisms’ inactivation (Lee et al., 2013). According to Pokhrel et al. (2017), this result occurs because the exposure of samples in temperatures above 50°C causes the weakening of the cell membrane and the death of microorganisms. Food composition and ultrasound operation conditions (e.g., frequency, time, temperature and volume) can affect microbial load reduction (Chandrappa et al., 2012). Therefore, different results are observed, and the treatment conditions must be studied to adapt the ultrasound technology to the processing industry.

3.2. pH, total soluble solids, and total titratable acidity

All treatments have maintained pH and total titratable acidity values in comparison to the control (p > 0.05). However, there was a statistical difference in the total soluble solid content (p ≤ 0.05) between the samples (Table 1). The mean pH (3.3 ± 0.05) of the samples has indicated acid medium. In addition, pH and acidity are quality indicators associated with sensory qualities of juice, like aroma and flavor. Therefore, the maintenance of these parameters is desired after conservation treatments. Ultrasonic treatments applied at 40°C for 10 min, and at 50°C for 15 min, enabled significant total soluble solid reduction in comparison to untreated juice samples (p > .05). However, these treatments did not significantly differ from other ultrasound application conditions and thermal treatment. "Brix represents the total soluble solids dissolved in water – these solids comprise sugars, proteins and free acids. Total soluble solid reduction may be associated with organic acids bound to other molecules or to sugars' transformation after ultrasound treatment application (Garcia-Noguera et al., 2010).

A study performed by Tomadoni et al. (2017) showed that ultrasound (40 kHz, 20°C for 10 or 30 min) or pasteurization (90°C for 60 sec) treatments did not change the titratable acidity and total soluble solids of strawberry juice in comparison to untreated samples. Ultrasound (40 kHz, 25°C for 15, 30 or 60 min) and heat treatments (30 or 60 sec at 90°C) applied to mango juice did not cause statistically significant changes in total soluble solids and total acidity values in comparison to the control samples (Santhirasegaram et al., 2013). Cassani et al. (2020) performed a study with ultrasound (40 kHz, 20°C, 15 and 30 min) on prebiotic-rich strawberry juice and observed no effect on the total soluble solids or total acidity of the samples.

Ultrasound treatments at different operation conditions did not lead to any pH, total soluble solids, and total acidity losses in comparison to untreated juice samples in previous studies with strawberry (25 kHz, 20°C, 15, and 30 min; Bhat & Goh, 2017), carrot (24 kHz, 50°C, 54°C, and 58°C, 0 to 10 min; Pokhrel et al., 2017; 20 kHz, 4°C, 0 to 10 min; Chen et al., 2019), kiwifruit (25 kHz, 0 to 16 min; Wang et al., 2019a), mandarin (19 kHz, 50°C, 36 min; C. X. Cheng et al., 2020); red watermelon and yellow watermelon (26 kHz, 4 to 16 min; Yikmış, 2020). The results observed among these studies are associated with treatment conditions, which did not cause any damage to the structure of these parameters and it suggests that ultrasound is an alternative for preserving the physical-chemical parameters of juices.

3.3. Ascorbic acid, anthocyanins, total phenolic compounds and antioxidant capacity

Ascorbic acid contents have decreased by 17–23.6% after treatment application. Juice samples treated by thermal treatment presented higher ascorbic acid decrease (23.62%) than the untreated juice. The ultrasound treatment application at 25°C for 5 min was statistically similar to that of the control juice (p > .05) (Table 2). Ascorbic acid is one of the indicators widely used to assess the overall nutrition quality of different food types. Ascorbic acid is the least stable among all vitamins since it is easily destroyed during processing or storage due to exposure to heat, light and oxygen (Cassani et al., 2020; C. X. Cheng et al., 2020). Moreover, processing time may favor the degradation of this nutrient during thermosonication (Wang et al., 2019a, 2019b). Ultrasound application in fluid systems leads to the formation of free radicals. Excessive OH− generation by cavitation may induce ascorbic acid oxidation and reduce its content (Lee et al., 2013). These mechanisms may justify
El aporte de la capacidad antioxidante fue observado en el presente estudio, como tratamiento temperatura o tiempo incrementado. Resultados similares fueron observados en frutas de manzana (Abid et al., 2014), mango (Santhirasegaram et al., 2013), mandarina (X. Cheng et al., 2020), y sandía (Yılmaz, 2020) en procesos de prelación. Sin embargo, la adición de una solución antioxidante es un proceso答应 adversidad en preservar estos compuestos que se mantienen con tratamiento térmico de las condiciones y condiciones ambientales. Los resultados de los primeros de las muestras han demostrado que la actividad antioxidante ha disminuido (p < 0.05) en comparación con el tratamiento térmico (control), como se muestra en la Tabla 2. Las muestras de frutas tratadas con ultrasonidos / tratamiento térmico han mostrado la actividad antioxidante más alta (30,5%). Tratamientos ultrasonicos también han reducido la actividad antioxidante en las muestras analizadas. Sin embargo, pueden ser más eficaces en preservar estos compuestos que se mantienen con tratamiento térmico. Los antioxidantes son pigmentos naturales que se encuentran en las frutas; tienen actividad antioxidante y contribuyen al color de los alimentos. Sus colores varían desde rojo, púrpura, hasta azul (Finley et al., 2018). La degradación de antioxidantes puede ser asociada con reacciones de oxidación promovidas por la formación de radicales libres durante termocondicionamiento (Tiwari et al., 2008), así como con su exposición a factores como alta temperatura y acidez acídica (Finley et al., 2018).

La fruta de fresa sometida al tratamiento térmico presentó una disminución total de compuestos fenólicos (19%) y antióxido capacidad (21,1%). Los demás tratamientos fueron más eficientes en preservar estos parámetros en el sample (Tabla 3). El rango 90°C de 60 segundos se espera que los compuestos de la actividad antioxidante disminuyan o aumenten, respectivamente. En el sample del presente estudio, los compuestos fenólicos, en adición a los compuestos ascorbic y antioxidantes, se mantienen en relación con la actividad antioxidante.

Los resultados reportados por X. Cheng et al., (2014), los cuales se obtuvieron de la fruta de fresa purificada (90°C de 60 seg) prelación total de compuestos fenólicos de reducción de 13.7%, diferentes muestras sometidas a ultrasonido (p <40°C para 10, 20 or 30 min), retenidas estas compuestos en una manera más satisfactoria. Similar a ellos, la fruta de sandía con fruta de fresa y fruta de fresa pasteurizada también se demostró que la temperatura de prelación (90°C para 30 seg) promovió la disminución de total de compuestos fenólicos y actividad antioxidante de muestras; ultrasonido (26 kHz, 4 a 16 min) mejoró estos parámetros en todas las condiciones evaluadas (Yılmaz, 2020). Según otros estudios, las frutas sujetas a ultrasonido tratamiento presentaron mayores compuestos fenólicos en comparación con los tratamientos de prelación.

The total phenolics of kasturi lime juice sonicated (25 kHz, 20°C) increased from 263.8 to 272.0 and 336.0 mg Gallic acid equivalents/g after 30 and 60 min of processing,

| Table 2. | Medias ± desviación estándar del ácido ascórbico, antocianinas, compuestos fenólicos y capacidad antioxidante del jugo de fresa sometido a diferentes tratamientos de conservación. |
| --- | --- |
| **Treatment** | **Ascorbic acid (mg/100 mL)** | **Anthocyanins (mg/100 mL)** | **Phenolic compounds (mg/100 mL)** | **Antioxidant capacity (%)** |
| Control | 99.9 ± 3.4 | 139.9 ± 3.5 | 116.3 ± 3.7 | 79.9 ± 3.6 |
| Thermal treatment | 76.3 ± 1.2 | 97.3 ± 3.9 | 93.2 ± 6.6 | 63.8 ± 4.1 |
| US 5 min (25°C) | 97.9 ± 3.9 | 124.2 ± 3.0 | 116.9 ± 9.6 | 80.0 ± 4.4 |
| US 10 min (25°C) | 78.0 ± 5.6 | 118.4 ± 6.2 | 112.0 ± 1.6 | 74.6 ± 2.6 |
| US 15 min (25°C) | 79.7 ± 4.3 | 117.8 ± 5.3 | 118.1 ± 8.9 | 73.0 ± 5.9 |
| US 5 min (40°C) | 87.7 ± 7.3 | 121.8 ± 4.1 | 104.7 ± 5.9 | 78.5 ± 2.5 |
| US 10 min (40°C) | 77.1 ± 1.0 | 114.0 ± 4.4 | 112.1 ± 5.9 | 81.2 ± 3.0 |
| US 15 min (40°C) | 82.7 ± 1.4 | 111.3 ± 1.7 | 105.7 ± 3.1 | 79.5 ± 1.3 |
| US 5 min (50°C) | 82.9 ± 3.1 | 104.9 ± 6.9 | 109.9 ± 9.2 | 79.5 ± 3.9 |
| US 10 min (50°C) | 81.6 ± 1.7 | 103.4 ± 6.9 | 107.9 ± 0.8 | 79.5 ± 8.7 |
| US 15 min (50°C) | 80.9 ± 2.2 | 110.3 ± 3.4 | 110.5 ± 2.1 | 75.6 ± 4.5 |

*Medianas seguidas de la misma letra en la misma columna no difieren según la prueba de Tukey (p > 0.05) por triplicado. US: Ultrasonido. Tratamiento térmico: 90°C durante 60 segundos.
respectively; the time of processing improved in 16.4% (30 min) and 35.8% (60 min) the antioxidant capacity of juice (Bhat et al., 2011). The time of processing with ultrasound (20 kHz, 4 to 16 min) also had a positive effect on total phenols and antioxidant activity, and it increased on an average of 34.9% and 25.8%, respectively, these parameters on strawberry juice (Wang et al., 2019b). Similarly, ultrasound treatment (20 kHz, 4 to 16 min) enhanced the total phenolic compounds and the antioxidant capacity in kiwifruit juice after all conditions of the treatment; the sample sonicated for the longer time increased on 108.6% the content of total phenolic compounds and the antioxidant capacity in sample sonicated for 16 min differed from 28.4% (control sample) to 45.5% (Wang et al., 2019a).

The results obtained by Tomadoni et al. (2017) evidenced that ultrasound (20°C, 40 kHz, 10 and 30 min) did not affect the antioxidant compounds or the antioxidant capacity of strawberry juice, and these parameters in unprocessed juice were lower than in processed samples. Yılmaz et al. (2019) observed that the thermosonication process to quince juice had positive effects on phenolic compounds and attributed these improvements to the disintegration of the cell wall by the pressure created by cavitation during ultrasound application and the binding of hydroxyl radical. These results indicate that cell wall breakdown may favor the release of bioactive compounds with antioxidant power, such as phenolic compounds and ascorbic acid. However, the impact of ultrasound treatment depends on the food raw material and the conditions applied (e.g., frequency, time, temperature and volume). High temperatures or long-time exposure to conservation treatments can degrade bioactive compounds such as phenolic compounds. However, according to X. F. Cheng et al. (2014) and Tomadoni et al. (2017), the inclusion of a bleaching step had a positive effect on phenolic compound content and antioxidant capacity preservation.

### 3.4. Color

Samples subjected to sonication at temperatures higher than 40°C presented significant \( L^* \) increase (\( p \leq 0.05 \)) in comparison to the control (Table 3). However, the samples treated at 40°C (for 5 and 10 min) and 50°C (for 5 min) did not differ from the thermally treated or controlled samples. Parameter \( L^* \) is linked to the product brightness perception; thus, increased \( L^* \) indicates increased luminosity. Samples subjected to an ultrasound treatment at 50°C (for 10 and 15 min) presented a brightness increase by 12.9% in comparison to untreated strawberry juice. \( L^* \) increase may happen due to particle precipitation and make the medium brighter (Abid et al., 2014). Bhat and Goh (2017), Tomadoni et al. (2017), and Cassani et al. (2020) did not observe changes in the brightness of strawberry juice subjected to ultrasound treatments (25 or 40 kHz; 20°C; 10, 15 and 30 min). However, Rojas et al. (2016) sonicated peach juice (20 kHz; 22 ± 3°C; 3, 6, 10, and 15 min) and observed that the samples became clearer as the treatment time increased. Carrot juice treated by Chen et al. (2019) increased the brightness in the first 2 min of treatment (20 kHz; 0.95 W/ml power). Yılmaz (2020) also found an increase in \( L^* \) parameter after treatment (26 kHz, 4, 8, 12 and 16 min) of yellow watermelon juice, but no difference was observed after the same conditions of treatment with red watermelon.

Hue angle (\( h^* \)) values have increased (\( p \leq 0.05 \)) as the treatment temperature and time increased (Table 3). This behavior enables samples’ hue to gradual change in a positive direction, as well as to acquire lighter shade than the natural one. The reduced anthocyanin content observed in the present study (Section 3.3) may justify samples’ loss of shade. Cassani et al. (2020) did not observe any effects in hue after application of ultrasound treatment (40 kHz; 20°C; 15 or 30 min) on prebiotic-rich strawberry juice. All treatments have maintained Chroma values; there was no statistical difference in browning index between treated and control samples (\( p > .05 \)). Chroma represents shade intensity. Assumingly, increased ultrasound treatment temperature and time have improved this parameter. However, only the samples subjected to ultrasound treatment at 50°C for 10 and 15 min did not show Chroma values statistically (\( p > .05 \)) similar to that of the control sample (Table 3). Browning index maintenance after treatment application may indicate a lack of browning reactions in yellowness components (\( b^* \)) (Wang et al., 2019b). In general, maintenance and increment of color were previously observed in watermelon juice processed with ultrasound (Yılmaz, 2020).

The total color difference (\( \Delta E \)) indicates the magnitude of the color change between treated and control samples. According to Tiwari et al. (2008), \( \Delta E \) values <1.5 indicate small color changes whereas \( \Delta E \) values between 1.5 and 3.0 represent different colors in the analyzed samples. Accordingly, thermal treatment and ultrasound treatments at 25°C led to small color differences in strawberry juice. The other operating conditions led to different color variations in the assessed samples (Table 3). The highest total color difference was observed in samples subjected to ultrasound treatment at 50°C for 10 and 15 min. However, only the most intense treatment has shown statistical difference in this parameter, based on the sensory acceptance analysis (Section 3.6). Cavitation can induce color changes due to chemical reaction acceleration, increased diffusion and dispersion rates, aggregate formation and particle degradation (Cruz-Cansino et al., 2015).

Color is an important quality parameter evaluated by consumers. Thus, the study of the impact of conservation processes in color coordinates is essential to guarantee that fruit juices have the expected characteristics.

### 3.5. Optical microscopy

Optical microscopy enabled observing different agglomerate types, as well as the arrangement of particles in juice samples (Figure 1). The control sample, as well as samples subjected to thermal treatment and to sonication at 25°C for 5 min kept their particles dense and aggregated. Samples became more transparent, and pulp particles became more dispersed in the serum as treatment temperature and time increased. Similar results were previously reported for other fruit juices, such as apple (Ertugay & Başlar, 2014), peach (Rojas et al., 2016), guava (Campoli et al., 2018), strawberry (Wang et al., 2019b) and kiwifruit (Wang et al., 2019a).

Juice pulp has fragments of plant tissues that have ruptured after preparation or conservation treatments and were dispersed in the serum, which, in its turn, comprises water and intracellular soluble components such as sugars, acids and minerals (Campoli et al., 2018). Results found in the present study, as well as in other studies, suggest that...
time and temperature increase in ultrasound processing favor cell wall breakdown and the consequent release of intracellular components, a fact that makes their microscopic appearance rarefied and transparent. Moreover, the pressure observed during cavitation processes reduces particles’ size and can increase fruit juice homogeneity and stability (Campoli et al., 2018; Ertugay & Başlar, 2014; Rojas et al., 2016). Although the disruption of juice cells after treatment can induce losses in nutritional content and color, due to the exposure of nutrients and pigments in the sample to oxygen (Rojas et al., 2016), the treatments with ultrasound proposed in the present study have proven to be efficient preserving nutritional parameters and color attributes evaluated.

3.6. Sensory acceptance analysis

All the participants \( n = 100 \) have reported consuming juices. The highest consumption frequencies reported by them corresponded to daily (48%) or weekly (41%) intake. Thermal and ultrasound treatments at 50°C for 5, 10, and 15 min were the treatments applied at this stage. There was
no statistical difference (p > .05) in color, aroma, appearance, consistency, taste and overall acceptance between the thermal-treated samples and the ones subjected to ultrasound treatment at 50°C for 5 or 10 min (data not shown). Figure 2 represents the classification of the parameters evaluated for each one of the samples, based on the 9-point hedonic scale. The color of juice samples subjected to pasteurization and ultrasound treatment at 50°C for 5 and 10 min was classified between I like it very much and I really like it. It is important to highlight that color is one of the main parameters influencing beverage purchasing (Priyadarshini & Priyadarshini, 2018). Maintaining this characteristic in products treated with new technologies is essential.

Parameters such as aroma, appearance and consistency scored from I moderately like it to I like it very much. Flavor scored from I slightly like it and I moderately like it. The highest acceptance rates obtained for consistency (84.0%), taste (79.1%) and overall acceptance (85%) were observed in samples subjected to ultrasound treatment at 50°C for 5 min (data not shown). In the present study, longer exposure time with ultrasound at 50°C affected negatively the sensorial parameters evaluated. The sample sonicated at 50°C for 15 min presented lower sensory acceptance indices for all the evaluated attributes (p ≤ 0.05). Color, aroma, appearance, consistency, and overall acceptance scored from I slightly like it to I moderately like it. However, taste was classified between I do not like or dislike it and I slightly like it. Moreover, taste and purchase intention presented the lowest acceptance indices (<70%). It is suggested that the reduction in the total solid content of the sample treated at 50°C for 15 min may have influenced the flavor of the juice. According to C. X. Cheng et al. (2020), ultrasound treatment can release bonding aroma components and cause changes in the aroma.

Positive results were observed by Tomadoni et al. (2017) and Cassani et al. (2020) in sensory analysis applied to strawberry juice, even in samples exposed to ultrasound treatment for 30 min (40 kHz, 20°C). Other studies have also mentioned good acceptance of apple juices or nectars treated at 20 kHz, amplitude of 60, 90 and 120 µm for 3, 6 and 9 min at 20°C, 40°C and 60°C (Šimunek et al., 2013) as well as 24 kHz, amplitude of 50 and 100 µm for 5 and 10 min at 40°C, 50°C and 60°C (Ertugay & Başlar, 2014). Sonicated watermelon juice samples were well evaluated even after 16 min of processing for the red variety and 8 min for the yellow variety (Yılmaz, 2020). However, Wong et al. (2010) performed acceptance tests with sonicated blackberry juice (at 25°C for 4, 8, 12, 21 or 32 min) and observed that juices treated for more than 8 min had negative taste evaluation – samples were described as having cooked and disagreeable taste.

These results can be explained by the fact that each food type has its own features, which can be positively or negatively affected by ultrasound treatment. Extended period contact with the ultrasound may form aromatic compounds or intensify those already present in the juice. The pressure observed in liquid samples during cavitation may favor undesirable flavors due to sample contact with the equipment for longer periods (Šimunek et al., 2013; Wong et al., 2010). Aromatic compound degradation can occur due to the extreme physical conditions inside the bubbles during cavitation. The formation or disappearance of the aromatic profile in juices and nectar juices after ultrasound treatment was evidenced (Šimunek et al., 2013; Jambrak et al., 2017; C. X. Cheng et al., 2020).

For this reason, it is important to study and compare different application conditions to adjust them and obtain a safe product, which maintains nutritional quality and is well accepted by consumers. The sample was treated with ultrasound at 50°C for 5 min pleased consumers positively.

4. Conclusion

Ultrasound is one of the emerging non-thermal methods that enhance quality and ensures safety, especially in food with heat sensitive, nutritional, sensory and functional characteristics.

Figure 2. Radar chart for attributes evaluated in the sensorial acceptance analysis of strawberry juice with 100 consumers. US – Ultrasound. Thermal treatment: 90°C for 60 seconds.

Figura 2. Gráfico de radar para los atributos evaluados en el análisis de aceptación sensorial del jugo de fresa en una muestra de 100 consumidores. US: Ultrasonido. Tratamiento térmico: 90°C durante 60 segundos.
Ultrasound application was a potential alternative to control microorganism growth in strawberry juice, as well as was more efficient in preserving nutritional quality. This may contribute to consumer demand for higher-quality juices. Ultrasound treatment at 50°C for 5 min emerged as a promising alternative to help preserve strawberry juice since it reduces the number of undesirable microorganisms, without impairing the physical-chemical and sensory features of the treated food.

Acknowledgments

We thank the Universidade Federal do Espírito Santo for the scholarship of the first and second author's and for project support.

Funding

The authors gratefully acknowledge the Foundation for the Support of Research and Innovation of Espírito Santo (FAPES) for financial support for project number [554/2015] approved on Edital Fapes no. [006/2014] and that allowed the acquisition of the equipment used in the present research.

Declaration of interest

The authors declare that there is no conflict of interest.

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