BRAZILIAN JOURNAL OF FOOD TECHNOLOGY

ISSN 1981-6723
Online version

ORIGINAL ARTICLE

Evaluating ultrasound pre-treatment as a tool for improving the process of a fermented beverage made from pineapple by-products

Avaliação do pré-tratamento com ultrassom como ferramenta para melhorar o processo de uma bebida fermentada a partir de subprodutos de abacaxi

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Cite as: Aguilar, K. (2022). Evaluating ultrasound pre-treatment as a tool for improving the process of a fermented beverage made from pineapple by-products. *Brazilian Journal of Food Technology*, *25*, e2021116. https://doi.org/10.1590/1981-6723.11621

Abstract

Fermentation has the potential of converting fruit by-products into value-added products via an efficient, sustainable, and low-cost process. Traditionally, Mexicans use pineapple residues to produce a fermented beverage called tepache. As this soft drink is increasingly consumed in restaurants, it is necessary to develop an effective and reliable process to yield a final product with desirable physicochemical properties. In this work, tepache was prepared using an ultrasound pre-treatment to enhance the fermentation process and improve the end-product quality. The ultrasound was provided by a probe (25 kHz, 400 W) submerged in pineapple preparations before fermentation. Characterization of physicochemical properties was performed on samples processed under different types of amplitude (20 and 100%) and sonication time (5 and 10 min). In all samples, the pH, acidity, and °Brix values were similar to those in commercial tepaches. On the other hand, microscopy revealed that 5 min of sonication induced positive changes in the suspended matter responsible for the physical stability of fruit beverages. The tepaches obtained with this method had color uniformity. Indeed, 5 min of sonication at the highest amplitude (16.34 kJ · cm^{-2}) augmented the soluble solids during the initial phases of fermentation. Moreover, the results from IR spectroscopy proved that ultrasound helped the ethanol release from yeasts. The maximum ethanol yield, calculated by model fitting, had a positive variation of 35%. These findings prove that ultrasound is capable to induce physicochemical changes useful for the industrial production of tepache.

Keywords: Tepache; Fermentation; Ultrasound processing; Appearance; Ethanol yield.

Resumo

A fermentação tem o potencial de converter subprodutos de frutas em produtos de valor adicional por meio de um processo eficiente, sustentável e de baixo custo. Tradicionalmente, os mexicanos usam resíduos de abacaxi para produzir uma bebida fermentada chamada tepache. Como este refrigerante é cada vez mais consumido em restaurantes, é necessário desenvolver um processo eficaz e confiável para obter um produto com propriedades físico-químicas desejáveis. Neste trabalho, o tepache foi preparado por meio de um pré-tratamento com ultrassom, a fim de aprimorar o processo de fermentação e melhorar a qualidade do produto acabado. O ultrassom foi fornecido por uma sonda (25 kHz, 400 W) submersa em preparações de abacaxi antes da fermentação. A caracterização das propriedades físico-químicas foi realizada em amostras processadas sob diferentes condições de amplitude (20 e 100%) e tempo de sonicação (5 e 10 min). Em todas as amostras, os valores de pH, acidez e °Brix foram semelhantes aos dos tepaches comerciais. Por outro lado, a microscopia revelou que 5 min de sonicação induziu mudanças positivas na matéria suspensa responsável pela estabilidade física das bebidas de frutas. De fato, os tepaches obtidos com este método apresentaram uniformidade de cor e observou-se que o tempo de sonicação de 5 min na amplitude mais alta (16.34 $kJ \cdot cm^{-2}$) aumentou os sólidos solúveis durante as fases iniciais da fermentação. Além disso, os resultados da espectroscopia de IR provaram que o ultrassom ajudou a liberar etanol das leveduras. O rendimento máximo de etanol, calculado por ajuste de modelo, teve variação positiva de 35%. Esses achados comprovam que o ultrassom é capaz de induzir alterações físico-químicas úteis para a produção industrial de tepache.

Palavras-chave: Tepache; Fermentação; Processamento com ultrassom; Aparência; Rendimento de etanol.

Highlights

- Traditionally, Mexicans use pineapple residues to produce a fermented beverage called tepache.
- In this work, tepache was prepared using an ultrasound pre-treatment to enhance the fermentation process and improve the end-product quality.
- 5 min of sonication induced positive changes in the suspended matter and color uniformity of tepache.
- Sonication at 16.34 $kJ \cdot cm^{-2}$ augmented the soluble solids during the initial phases of fermentation and increased the maximum ethanol yield up to 35 %.
- Ultrasound is capable to induce physicochemical changes useful for the industrial production of tepache.

1 Introduction

Pineapple waste management is a current critical for the agroindustry, as this is an abundant residue (Romero-Luna et al., 2017; Seguí & Fito, 2018). Therefore, several approaches have been proposed to reutilize this material for the generation of different value-added products (Seguí & Fito, 2018; Ketnawa et al., 2012), which is part of the current effort to convert the food production to a circular economy model. Nevertheless, these approaches are very sophisticated and need long processes involving operations such as drying, size reduction, extraction, filtration, and other separation processes, resulting on expensive procedures that require high-cost investments (Li et al., 2018).

Fermentation is an ancient technology which has been used to produce beverages from fruit surpluses and by-products. This procedure has prevailed for centuries in many cultures owing to its simplicity and use of local resources. For instance, Mexican families have fermented pineapple residues to prepare a soft drink, commonly known as tepache. This beverage is currently considered as a gourmet-like refreshment in specialized restaurants, which has increased its added value. Therefore, the development of efficient tepache production process at industrial scale represents a commercial opportunity.

In the artisanal process of tepache, the mix of pineapple by-products, water, and brown sugar (piloncillo, rapadura), is led to ferment at room temperature in glass or wooden tanks covered with cheesecloth. After a few days a refreshing, pleasant, sweet beverage with an alcohol content of less than 1% by volume is produced (Corona-González et al., 2013; Moreno-Terrazas, 2005). The final physicochemical properties of tepache are closely related to the microbiological activity undergoing during the fermentation process. *Saccharomyces cerevisiae* is the predominant microorganism, contributing to the unique organoleptic properties of tepache because of its capability to produce ethanol and CO_2. On the other hand, the essential bittersweet taste of tepaches results from the activity of acetic and lactic bacteria (Corona-González et al., 2013; Moreno-Terrazas, 2005).

As with most of traditional fermented foods (Misihairabgwi & Cheikhyoussef, 2017), inoculation is spontaneous, largely uncontrolled, in which microorganisms associated with the raw food material and processing environment act as inoculants. Thus, the overall process of tepache is time-consuming and inefficient. In contrast, the modern fermentation industry demands highly competitive and innovative processes to yield quality products. In this context, the combination of current or emerging food production technologies with traditional technologies has the potential to provide sustainable production solutions in a modern, efficient, and profitable fashion. For instance, some technologists have proposed the combination of physical treatments such as microwave or pressure cooking paired with enzymatic saccharification to enhance the extractability of fermentable sugars from pineapple peels to produce wine and vinegar (Roda et al., 2014).

Ultrasound processing (sonication) has been proposed to improve fermentation processes (Ojha et al., 2017). Power ultrasound (20-100 kHz) has demonstrated a growth stimulant effect on microorganisms depending on the intensity and the frequency of ultrasound. Appropriate sonication doses may enhance mass transfer and cell permeability, leading to a better fermentation efficiency and higher production rates (Dai et al., 2017; Ojha et al., 2017; Sulaiman et al., 2011). However, a severe use of sonication can lead to death of fermenting microorganisms (Akdeniz & Akalin, 2022). In addition, sonication can induce physical changes associated with sensory properties such as color and turbidity. Ultrasound propagation enhances transport phenomena and has shown to promote changes in the microstructure of fruit juices, producing physical stable systems with consistent cloudiness and color (Rojas et al., 2016; Campoli et al., 2018).

Therefore, the present work aimed to evaluate the effect of ultrasound pre-treatments on physicochemical parameters of preparations of pineapple cores and peels with sugared water. The selected parameters were measured before, during or after fermentation to elucidate the possible mechanisms induced by ultrasound pre-treatments throughout the process of tepache.

2 Materials and methods

2.1 Raw materials

Pineapples (*Anannas cosmosus* L.) var. Cayena Lisa were acquired in a local market (Jalapa-Enriquez, Mexico). The fruits were transported to Mexico City and stored for five days. The temperature during transportation and storage was not controlled. The crown was discarded, and the fruits were washed exclusively with tap water. In addition, pineapples were manually peeled and cored. Peels with rests of pulp and cores were grounded using a stainless-steel blender. This material was packed in plastic bags and stored inside a domestic freezer until use. Brown sugar (produced by Xiugar S. de R.L. de C.V., Mexico) and potable water were used as ingredients.

2.2 Preparation of samples

A mix, consisting of 250 g of thawed pineapple solids and 500 g of ~~a~~ sugar solution (15% w/w), was prepared. This mix was submitted to sonication or not sonicated (control treatment). Aliquots of these mixes were taken for analysis and named as "extracts". After that, portions of 450 g of each sonicated mix were weighted inside 500-mL Erlenmeyer flasks, covered with rubber stoppers, and placed inside a water bath at 24 °C for 72 h. After that, the rough solids were removed by cloth filtration and the filtrate was considered as tepache (Figure 1).

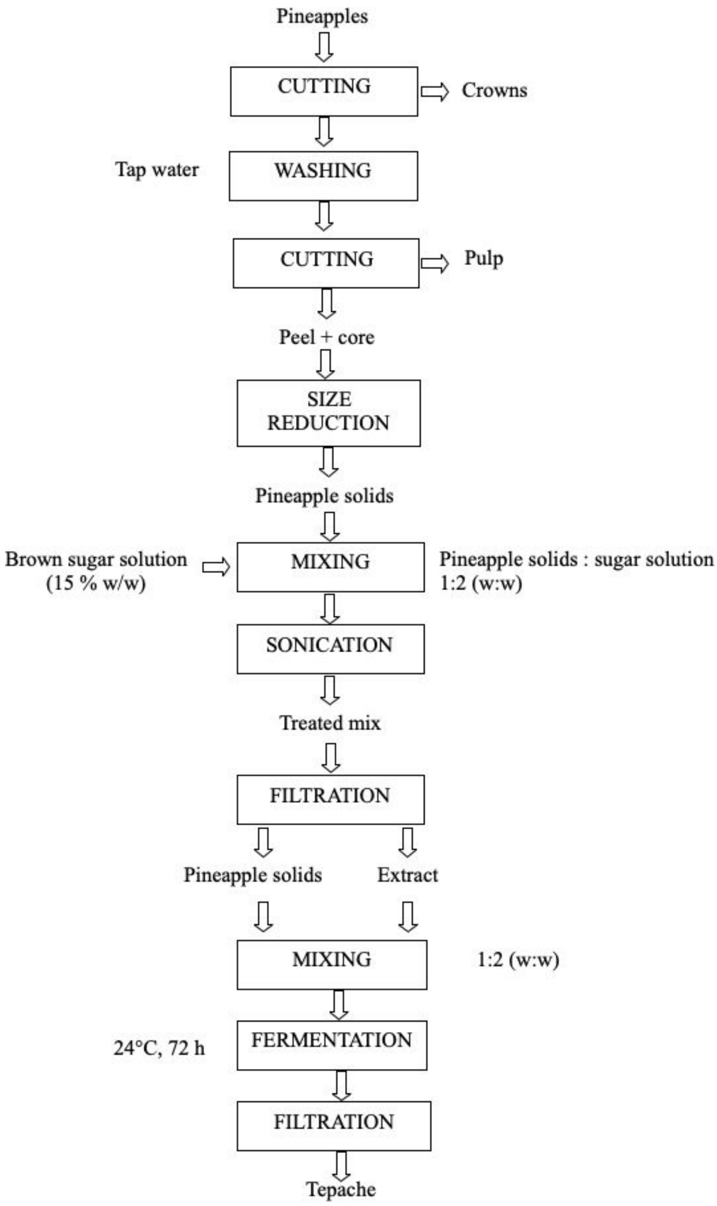

Figure 1. Process diagram of tepache.

2.3 Ultrasound (US) processing

US processing was performed using a sonication probe UP400S (Hielscher, Teltow, Germany) with a tip of 7 mm in diameter. The equipment operates at 25 kHz with a nominal power of 400 W (300 W × cm^{-2}). The tip was introduced into the sample (750 mL) at 10 mm from the surface. The amplitude of the oscillatory system was set at 20% or 100%. The real power (P in W) as well as the ultrasound intensity (UI in W×cm^{-2})

provided by the equipment were determined by the calorimetric method of Tiwari et al. (2008). $P_{20\%} = 7.48$ W, $UI_{20\%} = 19.40$ W × cm^{-2}, $P_{100\%} = 20.96$ W, $UI_{100\%} = 54.46$ W × cm^{-2}. The ultrasonic energy input (UE in J × cm^{-2}) was computed as in Equation 1:

$$UE = UI \times sonication\ time \quad (1)$$

After sonication, the solid fraction was separated by cloth filtration. Aliquots of these filtrates (extracts) were taken for analysis.

2.4 Experimental design

Two processing variables were considered, amplitude percentage and sonication time. For amplitude, two conditions were assayed, 20 and 100%. For sonication time, the assayed conditions were 5 and 10 min. Non-sonicated samples were considered as the control treatment. Experiments were replicated twice, and the order of treatments was randomly selected.

2.5 Yeast sonication test

With the aim to enquire into the effect of ultrasound on *S. cerevisiae*, a rapid test was conducted as follows. In fact, 11g of commercial bread yeast Tradi-Pan (SAFMEX, S.A. de C.V. & FERMEX S.A. de C.V., State of Mexico, Mexico) were dispersed into 700 mL of purified water. In addition, the suspensions were processed by ultrasound (at 20 and 100% of amplitude) for 5 min. Non-sonicated suspensions were used for control. All samples were analyzed by ATR-FTIR~~,~~ as described below.

2.6 Physicochemical analysis

Both, extracts, and tepache samples, were analyzed to determine their pH, acidity, soluble solids as Brix degree, and absorption spectra in the visible region. For these measurements, the aliquots were previously filtrated with paper Whatman No. 2. The pH was known with a pH meter ORION 250 A (Thermo Fischer Scientific, Beverly, MA, USA). The Brix degree were measured by using an Abbe-3L refractometer (Bausch & Lomb, NY, USA). The total titratable acidity was determined according to the Official Method (Association of Official Analytical Chemists, 2007). The visible absorption spectra, from 400 to 700 nm, were obtained with a GENESYS 2 spectrophotometer (Spectronic Instruments Inc., NY, USA). All measurements were carried out in duplicate.

2.7 Microscopy

Both, extracts, and tepache samples, were observed by means of light microscopy. For this purpose, the aliquots were placed on viewing slides, upon which coverslips were gently placed. An optimal microscope (Olympus BX45, Olympus Optical Co., Ltd. Tokyo, Japan) was used. Micrographs were taken with an AxioCam FRC 5s (Carl Zeiss GMBH, Oberkochen, Baden-Wüttemberg, Germany) digital camera, equipped with a specialized software (Zen version 1.0, Carl Zeiss GMBH) to record and process the images. Selected micrographs at 10× or 50× are presented.

2.8 ATR-FTIR spectroscopy

The chemical changes promoted by US were further studied by Fourier Transform Infrared (FTIR) analysis. The infrared spectrum was obtained using a Perkin Elmer spectrophotometer (Spectrum 100, Perkin Elmer, Waltham, MA, USA) equipped with a crystal diamond universal ATR sampling accessory. The mirror velocity was 0.4 cm/s. Before each measurement the ATR crystal was carefully cleaned with ethanol. A spectrum of the empty cell was used as background. For each sample, the spectrum represented an average of twelve scans with 1/cm resolution. Spectra were baseline-corrected at 4000-400/cm.

2.9 Fermentation kinetics

During the fermentation process, the ºBrix, the ethanol and the carbon dioxide were monitored. The soluble solids were analyzed by refractometry in a small aliquot (~5 drops) taken from the flask. The production of ethanol and CO_2 was evaluated by determining the amount of these gases in the headspace. These parameters were quantified with two gas sensors (CO_2-BTA and ETH-BTA) provided by Vernier Software & Technology (Beaverton, OR, USA). The sensors were plugged to a Vernier LabPro® interface connected to a Voyage 200 calculator (Texas Instruments, Dallas, TX, USA). The ethanol and CO_2 concentrations were measured at least twice.

The production of ethanol in the headspace was fitted to a semi-empirical model (Equation 2), analogous to the equation proposed by Ibarz & Augusto (2015) for microbial growth:

$$E(t) = (K_1 \times exp[K_2 \times t]) / (K_3 + exp[K_2 \times t]) \quad (2)$$

Here, $E(t)$ represents the ethanol concentration in the headspace (%), as a function of time (t in h). K_1 represents the maximum ethanol yield (E_∞), K_2 is proportional to the reaction kinetic constant (k), and K_3 (Equation 3) describes the relative difference between the initial and final ethanol concentration values, for a specific initial value:

$$K_3 = (E_\infty - E_0) / E_0 \quad (3)$$

2.10 Statistical analysis

The average of the measurements was considered as a single data. Means and standard deviations of the two replicates were calculated and reported. A two-way Analysis of Variance (ANOVA) was performed to evaluate the effects of ultrasound processing and fermentation on the physicochemical parameters. The production of ethanol in the headspace as a function of time was fitted to Equation 2 by means of a non-linear regression with the STATGRAPHICS Centurion v. 18 statistical software. The data from the two replicates were considered, as well as a confidence level of 95%. The goodness of fitting was evaluated graphically and by the adjusted R^2.

3 Results and discussion

3.1 Physicochemical analysis

To explore the mechanisms induced by the ultrasound pre-treatments on the preparations submitted to fermentation~~,~~ and their consequences on the tepaches obtained, different physicochemical attributes were evaluated (Table 1). The initial pH value was within the interval between 3.5 and 5, as recommended to obtain high acceptance of the final product (Corona-González et al., 2013). In all cases, the titratable acidity and the pH value of the extracts were not significantly altered by US processing ($p > 0.05$), but the pH value decreased after fermentation ($p < 0.05$). This change was expected due to the formation of CO_2 and organic acids produced as a result of the metabolism of tibicos (Rubio et al., 1993). The titratable acidity was not altered ($p > 0.05$), probably due to the elimination of CO_2 for this test. The final pH was closed to the interval between 3.10 and 3.44 reported by Moreno-Terrazas (2005) for commercial tepaches.

The ºBrix are usually related to the sugar content in fruit beverages. For all the extracts analyzed, this value was minor to the sugar percentage in the solution added to formulations. This preliminary reduction of sugars in the solution could be a consequence of an osmotic mass transfer of solids through the pineapple solids, possibly meanwhile the mixes were handled. This phenomenon has been described for fruit pieces placed in sugared solutions, thus creating a gradient of concentration of this solute (Chandra & Kumari, 2015). According to Corona-González et al. (2013) the optimal sugar concentration in the solution added should be 10-15% to obtain a tepache with acceptable sensorial score. After fermentation, the soluble solids content was reduced, as expected due to the consumption of sugars by yeasts (Rubio et al., 1993). The final value in the tepaches was close to 12 ºBrix, fitting to consumer preferences (Moreno-Terrazas, 2005).

Table 1. Physicochemical parameters of the extracts obtained after US processing and the corresponding tepaches obtained after 72 h of fermentation.

Treatment	UE (kJ × cm^{-2})	pH	Titratable Acidity (g citric acid/100 mL)	Total Soluble Solids (°Brix)
Extracts				
Control	0	3.68 ± 0.07^b	0.23 ± 0.08^a	13.64 ± 0.62^b
20% amplitude, 5 min	5.82	3.67 ± 0.01^b	0.32 ± 0.05^a	13.67 ± 0.47^b
20% amplitude, 10 min	11.64	3.71 ± 0.01^b	0.30 ± 0.05^a	13.54 ± 0.09^b
100% amplitude, 5 min	16.34	3.73 ± 0.01^b	0.37 ± 0.15^a	13.67 ± 0.19^b
100% amplitude, 10 min	32.68	3.72 ± 0.04^b	0.44 ± 0.19^a	12.13 ± 1.13^b
Tepaches				
Control	0	3.45 ± 0.06^a	0.31 ± 0.01^a	12.55 ± 0.07^a
20% amplitude, 5 min	5.82	3.49 ± 0.06^a	0.35 ± 0.05^a	12.35 ± 0.59^a
20% amplitude, 10 min	11.64	3.35 ± 0.04^a	0.32 ± 0.01^a	12.35 ± 0.12^a
100% amplitude, 5 min	16.34	3.41 ± 0.02^a	0.37 ± 0.15^a	12.75 ± 0.02^a
100% amplitude, 10 min	32.68	3.41 ± 0.20^a	0.33 ± 0.04^a	12.58 ± 1.06^a

Different superscripts represent significant differences in a column. UE is the ultrasonic energy input, calculated by Equation 1.

3.2 Optical microscopy

Figure 2 presents micrographs of the extracts obtained after the softest and the most severe treatments compared to control. These liquids are composed of water, solved solids, suspended particles and microorganisms. In the non-processed extracts (Figure 2a) suspended particles and some fiber structures were observed. The suspended solids appeared finer when the mix was processed for 5 min (at 20% of amplitude) (Figure 2b). In contrast, the mixes treated at 100% of amplitude for 10 min showed rough suspended particles, possibly pineapple cell fragments (Figure 2c). It is possible that sonication had promoted changes in the microstructure and composition of the liquid suspension that will be converted into tepache. These changes may have been induced by different mechanisms, including plant cell damage and release of intracellular content, particle size reduction, disruption of the whole plant cells, polysaccharide size reduction and dispersion of constituents, as demonstrated by Rojas et al. (2016).

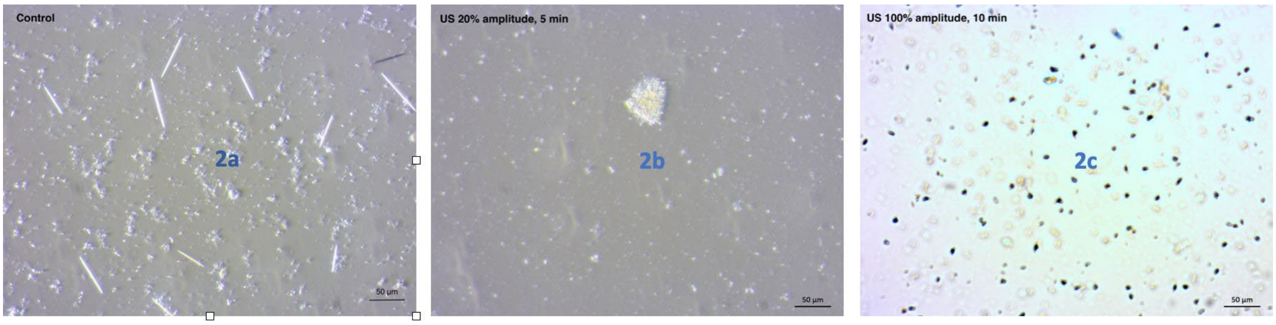

Figure 2. Micrographs of different extracts from pineapple preparations after sonication and control.

Besides, a conglomerate is observed in Figure 2a, likely tibicos. According to the literature (Romero-Luna et al., 2017; Rubio et al., 1993), tibicos are compact, whitish, or yellowish, translucent, or opalescent, gelatinous masses, of irregular shape and size. Furthermore, yeast shapes were found in all the tepache samples (Figure 3).

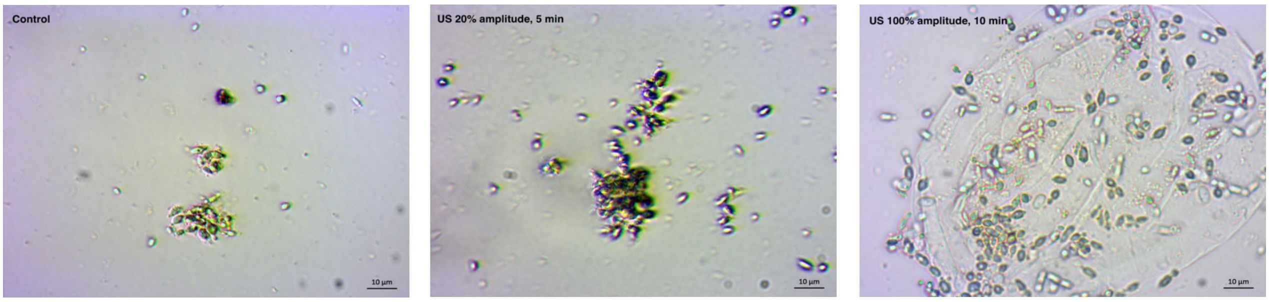

Figure 3. Micrographs of different tepaches obtained from sonicated preparations and control.

3.3 Visible absorption spectra

The visible absorption spectra of the tepaches obtained are presented in Figure 4. It is observed that they mostly absorb light between 400 and 450 nm, indicating the presence of yellow-brown pigments from brown sugar and pineapple. For all the samples, the spectrum followed the same pattern, meaning that sonication did not promote color changes in the liquid converted to tepache. However, by comparing the absorbance values at the same wavelength, some differences can be appreciated.

The absorbance at 420 nm is usually an indicator of the presence of brown pigments in fruit juices. Therefore, the media values and standard deviations of this parameter are represented in Figure 4. The tepaches from mixes sonicated for 5 min showed values similar to those in the controls and low deviations. On the contrary, the products from the preparations sonicated for 10 min exhibited great variations. These findings suggest sonication increasingly promotes changes in the soluble and non-soluble compounds of tepache, thus affecting its physical properties and appearance. These changes could be a consequence of structural modifications promoted by ultrasound. Rojas et al. (2016) demonstrated that ultrasound can trigger different mechanisms that determine the final physical properties of peach juice. The effect of US on the physical properties and visual appearance of tepache is interesting for the food industry and should be further investigated.

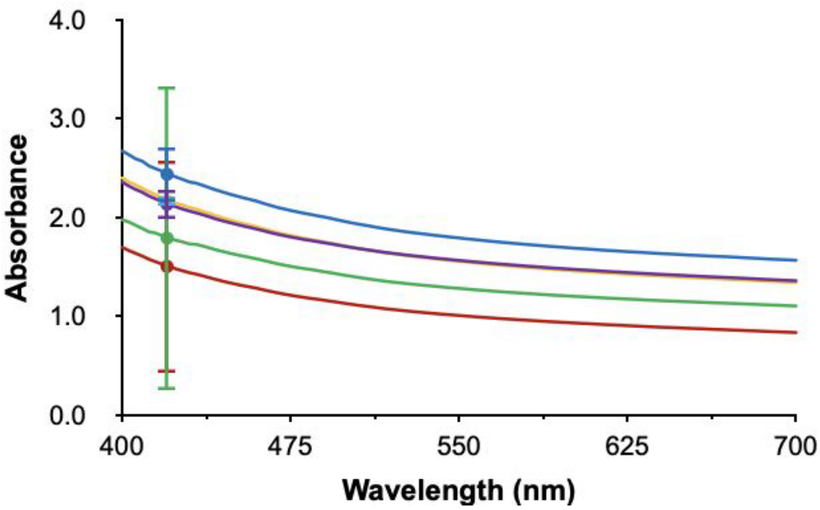

Figure 4. Colour attributes of the tepaches from mixes processed by US and control. Absorbtion spectra (lines), absorption at 420 nm mean data (dots) and standard variations (bars).

3.4 FTIR

The infrared spectra of the tepache samples were explored to gain insight on the chemical modifications induced by ultrasound pre-treatments (Figure 5a). The spectra of pure ethanol and distilled water were also obtained for comparison (Figure 5b). The broad band in the region between 2800-3800 cm^{-1} indicates the presence of H-bonded –OH groups in water. Moreover, tepaches spectra showed a double peak at 1050 cm^{-1}, which is characteristic of the C-O bond in alcohol molecules (Figures 5a, b). Other ethanol footprints were evident, such as the signals at 1200-1500 cm^{-1} and 2800-3000 cm^{-1} corresponding to alkyl groups.

Interestingly, the ethanol footprints were more pronounced in tepaches from mixes processed by ultrasound than in controls (Figure 5c). In fact, the rapid test with commercial yeast suspensions also showed an increase in these signals after US processing (Figure 5d). These results proved that US technology favors the releasing of ethanol from yeasts cells by promoting the exchange of materials between intracellular and extracellular, as declared by Dai et al. (2017).

Moderate sonication resulted on higher ethanol footprints (Figures 5c, d), as compared to the softest and the most intense treatments assayed. This finding is consistent with earlier studies. Sulaiman et al. (2011) observed that low intensity ultrasound (10% and 20% duty cycles) stimulated yeast growth in a lactose system, but the 40% duty cycle had an adverse impact on cell growth. Dai et al. (2017) reported different variations on the biomass content of a model system, depending on the intensity of US processing. They registered the highest increment in the biomass under moderate sonication conditions. Conversely, low levels of sonication resulted in a slow microbial growth, whereas severe treatments promoted microbial death.

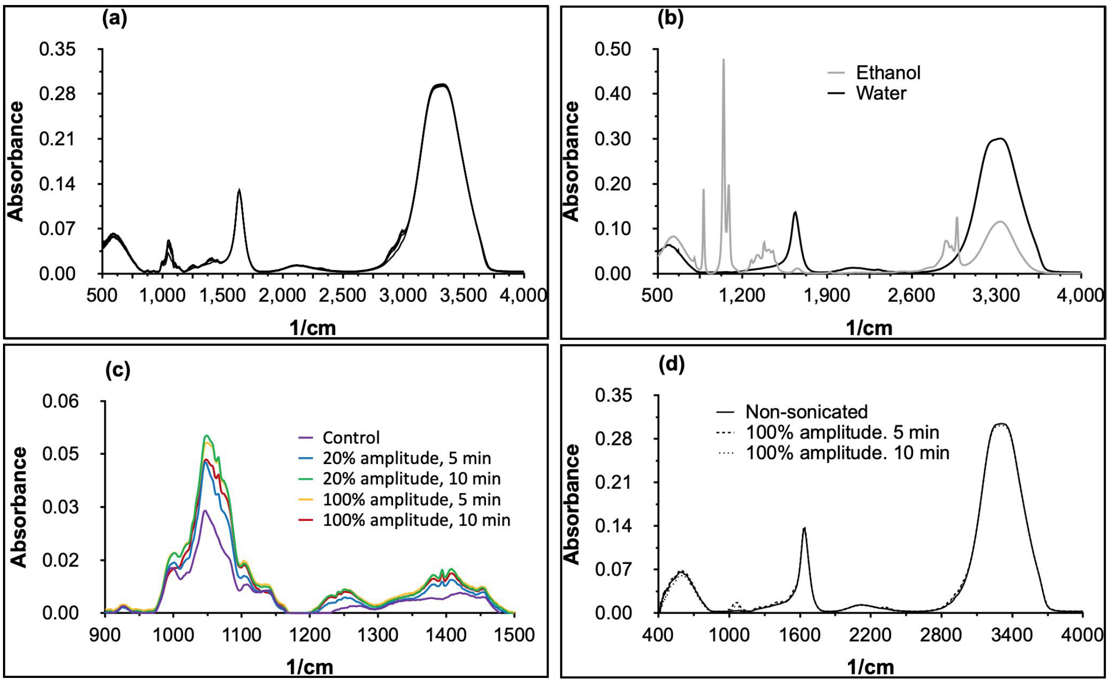

Figure 5. IR spectra of the tepaches from mixes processed by US and reference materials. (a) Tepaches and control (b) Water and ethanol (c) Tepaches and control (900-1500 1/cm) without the water band (d) Sonicated and non-sonicated yeasts suspensions.

3.5 Fermentation kinetics

The results obtained from physicochemical analysis, IR spectroscopy and visual inspection led to the conclusion that the better ultrasound pre-treatment for improving the process of tepache was at amplitude of 100% for 5 min. Therefore, the fermentation was exclusively monitored in mixes treated under those conditions and in non-sonicated mixes. The evolution of ethanol, carbon dioxide and º Brix is plotted in Figure 6.

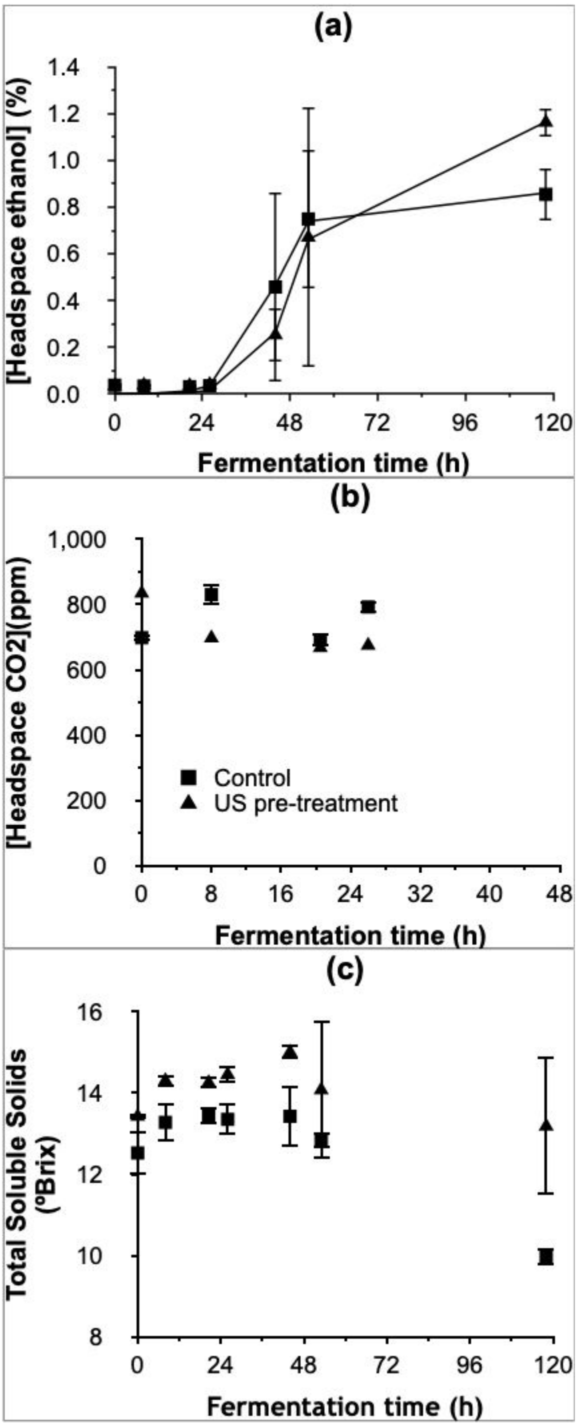

Figure 6. Evolution of metabolites (a) ethanol, (b) carbon dioxide, and (c) total soluble solids, during the fermentation for producing tepache, with and without US pre-treatment. Dots represent mean values, bars represent standard deviations, and lines represent fitted models.

The ethanol production kinetic was analog to the typical microbial growth, which has sigmoidal shape with three stages (Ibarz & Augusto, 2015) including lag phase or period of adaptation, exponential phase, and stationary phase. From Figure 6a, it seems that the lag phase lasted approximately 1 day, and the exponential phase started the second day. The lag phase can be confirmed by following the CO_2 in the headspace (Figure 6b), which was almost constant for the first day. After that, this parameter markedly increased, overpassing the upper limit of detection (5000 ppm), and indicating the beginning of the exponential phase.

Attending the evolution of the ºBrix (Figure 6c), three stages can be identified:

(1) The º Brix slightly increased. It means that more sugars like sucrose were solved in the media, probably as a consequence of a best solubilization during tempering;

(2) This parameter became practically constant in the control, suggesting an insignificant consumption of sugars by microorganisms. On the other hand, the soluble solids content in sonicated samples still increased in this period. A hypothesis to explain this behavior is that sonication promotes damage in the lignocellulose of pineapple fiber. Seguí & Fito (2018) reported an average of 103.6 g/L of fermentable sugars content in pineapple waste. However, these sugars are trapped by lignocellulosic material (Roda et al., 2014). Roda et al. (2016) proved that different physical methods (e.g. microwave heating and high-pressure cooking) may help to break this recalcitrant effect, improving the release of fermentable sugars after an enzymatic hydrolysis;

(3) The ºBrix was markedly reduced after 48 h, indicating that the consumption of sugars by microorganisms was the predominant mechanism.

The fermentation kinetics (Figure 6a) were fitted to Equation 2 and the resulting parameters are summarized in Table 2. The fitted models are also represented by lines in Figure 6a. As can be seen, this model provided a good fitting to the experimental data ($R^2_{adj} > 0.80$). The K_3 values can be related to the duration of the lag phase (Ibarz & Augusto, 2015). These results showed a wide variation since fermentation was only started with the natural microbiota present in the pineapple.

Table 2. Resulting parameters from the fitting of fermentation kinetics of tepache, processed or not by ultrassound, to the model of Ibarz-Augusto. $E(t) = (K_1 \times exp[K_2 \times t])/(K_3 + exp[K_2 \times t])$.

Treatment	K_1 (%)	K_2 (h^{-1})	K_3 (-)	R^2_{adj}
Control	0.861 ± 0.086	0.182 ± 0.086	2534.12 ± 318.28	0.8615
US pre-treatment (20% amplitude, 5 min)	1.165 ± 0.072	0.167 ± 0.004	5306.96 ± 1EXP-6	0.9288

The parameter K_2, proportional to the reaction kinetic constant, did not significantly increase with sonication at the conditions assayed (16.34 J×cm^{-2}). In this study, the medium was processed by sonication at the beginning of the microbial growth. Other authors have shown that the application of ultrasound at different stages of the curve influenced the fermentation kinetic (Dai et al., 2017). K_1 resulted 35% higher in average for the tepaches from mixes processed by ultrasound. This parameter represents the maximum ethanol yield concentration produced (Ibarz & Augusto, 2015). Therefore, it is confirmed that ultrasound processing enhances the ethanol production.

4 Conclusions

To produce the Mexican beverage called tepache on an industrial scale, ultrasound technology was proposed to improve the traditional process. Therefore, mixes of pineapple by-products, water, and sugar, were sonicated before fermentation. The results showed that ultrasound processing from 5.82 to 32.68 J·cm^{-2} in 500 mL samples triggered physicochemical mechanisms that could be desirable or undesirable during the production of tepache, depending on the exposure time and ultrasound amplitude. First, it was observed that sonication seemed to increasingly promote changes in the microscopic solids suspended in the aqueous phase, thus augmenting, or reducing the color variations between replicates. Furthermore, the results from the different physicochemical analyses suggest that moderate ultrasound pre-treatments (16.34 J × cm^{-2}) may help to release solutes from biological materials, such as sugars from pineapple tissues and ethanol from yeasts cells. These initial findings are interesting for the industrial production of tepache and should be further investigated to optimize the process.

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Funding: This research was funded by the Scholarship approved by the National Council of Science and Technology (CONACYT) for the Postdoctoral Stay of Dr. Karla Aguilar (CVU:258816). The experiments were performed in the Laboratory of Bioprocesses of the Iztapalapa Metropolitan Autonomous University.

Received: June 28, 2021; Accepted: May 09, 2022

Associate Editor: José Humberto de Oliveira Filho.