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Impact of High-Pressure Processing on the Physicochemical and Storage Stability of Tepache, a Pineapple By-product Fermented Beverage

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Abstract

Tepache is a functional fermented beverage made from pineapple by-products. It is rich in prebiotics and bioactive compounds. However, prolonged storage of the beverage can lead to further proliferation of microorganisms, eventually resulting in an undesirable taste and changes in nutritional composition. There is a need to extend the shelf life of the tepache without compromising its organoleptic and nutritional properties. In this study, the effect of high-pressure processing (HPP) on the physicochemical properties, microbial quality, and sensory characteristics of tepache was investigated. Samples were treated at 300, 450, and 600 MPa for 6 min and stored at 4 °C for 14 days. Total plate count, pH, total soluble solids, color, DPPH scavenging activity, and sensory evaluation were analyzed. HPP significantly reduced the microbial load, ranging from no viable cells to 6.00 $\log_{10}$ CFU/mL, with 600 MPa showing the highest efficacy. Tepache treated with HPP exhibited higher pH, total soluble solids, and sensory scores, which increased with higher pressure. The treatment maintained the color of the sample, but a slight decrease in b* was observed during storage. The DPPH scavenging activity was improved by the high-pressure treatment, and lower reduction was observed during storage compared to the control sample. The findings suggest that HPP at higher pressure is a suitable option to improve both the shelf life and quality of fermented beverages without compromising their antioxidative properties.

INTRODUCTION

Tepache is a popular traditional fermented beverage that originated from Mexico. It is made using pineapple peels, brown sugar, water, and some spices (optional) such as cinnamon and pepper. The mixture then undergoes natural fermentation at room temperature by native microorganisms originating from the ingredients and environment for 1 to 4 days [1,2]. The liquid from the fermented mixture is then filtered out and served as tepache. The organoleptic properties of tepache are contributed by its sweet taste, with low acidity, low ethanol, and the presence of other volatile compounds [3]. The fermentation process increases the level and bioavailability of bioactive compounds. The beverage is also rich in probiotics, ranging from various mesophilic aerobic bacteria, lactic acid bacteria, and yeasts [4,5]. Extending the shelf life of tepache presents several challenges. Uncontrolled storage conditions may allow microbial proliferation, which may adversely affect the composition and flavour of the beverage. Biochemical changes may still occur even under low-temperature storage due to the metabolic activity of psychrotrophic microorganisms. Tepache contains between 1.72 - 52.72 g/L ethanol [3], and the alcohol level may increase if fermentation continues during storage, raising concerns about its halal compliance.

High-pressure processing (HPP) is a non-thermal preservation technique often used to inactivate microorganisms while preserving the nutritional content of food. HPP treatment of fermented pomegranate juice at 600 MPa for 3 minutes successfully inactivated the microorganisms while preserving the physicochemical and bioactive compounds of the juice [6]. To date, studies on storage stability and the effects of high-pressure treatment on the quality of tepache are lacking. Therefore, this study investigates how the different HPP pressure affects the quality of tepache over 14-day storage at low temperatures.

MATERIALS AND METHODS

Materials

Ripe pineapples (MD2 variety) at ripening index 5 and brown sugar (MSM Prai Berhad, Malaysia) were purchased from local stores. All chemicals, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid (Sigma Aldrich, Gillingham, UK), and methanol are reagent grade.

Preparation and fermentation of tepache

Tepache was prepared according to Gutiérrez-Sarmiento et al. [2] with some modifications. Pineapples were washed and peeled. The pineapple peels, with a thickness of 1.5 to 2 cm, and cores were collected and washed under running tap water. The peels and cores were cut to a 3 x 3 cm size. Brown sugar (200 g) was dissolved in 1000 mL of distilled water. The peels and cores (350 g) were placed in a pre-sterilized plastic container, followed by the addition of the sugar solution. The lid was loosely closed, and the sample was fermented at 30 ℃ for 48 h. The fermentation was stopped when white foams were spotted on the surface of the tepache. The tepache juice was collected by straining out the peels and cores. The juice was then strained one more time. The tepache juice was packed in plastic bottles (150 mL).

High-pressure processing of tepache

The tepache was treated using a commercial-scale high-pressure processing (HPP) machine (Hiperbaric Wave 6500/120, N.C. Hiperbaric, S.A., Bargas, Spain) at 300, 450, and 600 MPa for 6 min, labeled as HP300, HP450, and HP600, respectively. The untreated tepache is considered a control. The tepache was stored for 14 days at 4 ℃. The samples were analyzed right after the HPP (Day 0) and on Day 14, while sensory evaluation was performed on Day 0 samples.

Characterization of tepache

Total plate count (TPC) was used to determine the viable microbial load in the tepache. Serial ten-fold dilutions were prepared from 10^{-1} to 10^{-10} in buffered peptone water. An Aliquot (0.1 mL) from each dilution was transferred onto the plate count agar. Duplicate plates were prepared for each dilution. The plates were incubated at 37 ℃ for 48 h. Colony forming units (CFU) were counted with plates with 25 to 300 colonies were considered for record [7]. The pH of the tepache was measured using a pH meter, while the total soluble solids were determined using a refractometer.

The colour of the tepache was measured using a chromameter. Tepache (50 mL) was filled into a small transparent container with a white background on the bottom. The L*, a* and b* values were recorded. Chroma was calculated as follows [8]:

$$\text{Chroma} = (a^{*2}+b^{*2})^{1/2} \quad (1)$$

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging activity was determined according to the method of Oikeh et al. [9] with some modifications. DPPH (24 mg) was dissolved in 100 mL of methanol to prepare a 0.1 mmol/L DPPH solution. Tepache or standard (ascorbic acid) (0.1 mL) at varying concentrations (5, 10, 15, 20, and 25 mg/mL) was added into a test tube containing 0.9 mL methanolic DPPH solution. The reaction mixture was kept in a dark room for 30 minutes, and the absorbance was measured at 517 nm. The inhibition percentage of DPPH was calculated using the following equation.

$$\text{DPPH radical scavenging activity (\%)} = [A_0 - A_1) / A_0] \times 100 \quad (2)$$

Where A_0 is the absorbance control (DPPH radical + methanol) and A_1 is the absorbance of the sample or standard. Sensory evaluation of tepache was performed by 30 untrained panelists using a 1 – 9 hedonic scale for scoring color, aroma, sweetness, sourness, aftertaste, and overall acceptability (1 as dislike extremely, 5 as neither like nor dislike, and 9 as like extremely). The fermentation was performed in two batches. All analyses were conducted in triplicate for each batch. Statistical analysis was performed using Minitab (ver. 22, Minitab Inc, Penn., USA). The data was analysed using one-way ANOVA to determine significant differences among treatment groups, and a t-test was used to analyze the difference between storage durations. The least significant differences were calculated using Tukey's test at a significant level ($p \leq 0.05$).

RESULT AND DISCUSSION

Table 1 shows the total plate count (TPC) of tepache following high-pressure treatment (Day 0) and 14 days of low-temperature storage. The untreated sample (control) had a high microbial count, suggesting that the fermentation process enhanced the probiotic content of tepache. The viable cell counts significantly decreased following the HPP treatment. Treatment at 300 and 400 MPa did not fully inactivate microorganisms, however, increasing pressure resulted in greater microbial inactivation, with the sample treated at 450 MPa (HP450) recorded colony-forming units of <10 x 10^2 CFU/mL. At 600 MPa, the microbe was sufficiently inactivated to be below the detection limit.

Previous authors [10] reported a TPC count of ≤10 CFU/mL in sea buckthorn juice treated at 500 MPa for 5 min. However, storage of the tepache led to a significant increase in the microbial count in all HPP-treated samples, while a slight decrease was observed in the control sample. Similarly, Pereira et al. [11] reported an increase in viable cell count after 42 days of refrigerated storage of fermented cashew apple juice. The increase in viable cell count in the HPP-treated tepache could be due to the survival of acid- and pressure-resistant spores that may have proliferated during the storage, while the slight decrease in microbial count in the control sample could be due to the reduced viability of acid-intolerant microorganisms.

Table 1. Total plate count of tepache treated at different high-pressure levels measured on Day 0 and 14.

Samples	Log_{10} CFU/mL	
	Day 0	Day 14
Control	7.75 ± 0.59^{aA}	7.53 ± 0.19^{aA}
HP300	4.00 ± 0.00^{bB}	6.00 ± 0.20^{bA}
HP450	$< 2.00 \pm 0.00^{cB}$	4.23 ± 0.34^{cA}
HP600	ND	3.24 ± 0.34^{dA}

Note: ND: non-detected. Values with different lowercase within the same column indicate significant differences across different samples ($p \leq 0.05$). Different uppercase letters within the same row indicate significant differences across storage durations for the same sample at $p \leq 0.05$.

Tepache is a highly acidic beverage. **Table 2** shows that the pH of Day 0 tepache ranged from 3.20 to 3.40, with the high-pressure-treated samples showing a slight increase in pH after the treatment. Nonetheless, no significant trend was observed between the pressures tested. These results are consistent with other studies that found pH values of 3.5 and 3.2 to 3.4 for tepache after 48 and 72 hours of fermentation, respectively [2, 12]. The accumulation of organic acids via the metabolic activities of microorganisms during fermentation could be the reason for the low pH.

Tepache was reported to contain 1.12–33.92 g/L lactic acid and 1.12–10.3 g/L acetic acid [3]. A slight decrease in pH was observed in all samples following storage of the tepache, with values ranging from 3.16 to 3.26, with the control sample having the lowest pH. The reduction in pH of the control and HPP-treated samples could be due to the continued microbial activities during the storage, leading to increased acidity. These findings were supported by the increased viable cell count in the stored samples. *Lactobacillus casei* is capable of producing acid even at low temperatures, thus increasing the lactic acid content [11]. The sample treated at 600 MPa (HP600) showed a high reduction in pH value. This could be partially due to the oxidation of vitamin C. The degradation of ascorbic acid leads to the formation of dehydro ascorbic acid which can be further hydrolyzed to 2,3-diketo-l-gulonate or oxidized to other acids such as L-threonic and oxalic acids [13].

Table 2. Changes in pH and total soluble solids of tepache following high-pressure processing (Day 0) and low-temperature storage (Day 14).

Samples	pH		Total soluble solids (°Brix)	
	Day 0	Day 14	Day 0	Day 14
Control	3.28 ± 0.02^{bA}	3.16 ± 0.01^{bB}	15.8 ± 0.61^{aA}	14.7 ± 0.21^{bB}
HP300	3.39 ± 0.05^{aA}	3.22 ± 0.01^{aB}	15.4 ± 1.06^{aA}	15.5 ± 1.38^{abA}
HP450	3.37 ± 0.03^{aA}	3.26 ± 0.03^{aB}	16.6 ± 0.33^{aA}	15.4 ± 1.14^{abA}
HP600	3.40 ± 0.05^{aA}	3.22 ± 0.02^{aB}	15.9 ± 0.50^{aA}	15.8 ± 0.39^{aA}

Note: Values with different lowercase within the same column indicate significant differences across different samples ($p \leq 0.05$). Different uppercase letters within the same row indicate significant differences across storage durations for the same sample at $p \leq 0.05$.

Tepache commonly contains sucrose (0.73 -101.87 g/L), glucose (1.28 - 21.78 g/L), and fructose (1.02 - 45.52 g/L) [3], derived from brown sugar and pineapple by-products. The total soluble solids (TSS) of tepache ranged from 15.4 to 16.6 °Brix. High-pressure processing has no significant effect on the total soluble solids (TSS) content of the tepache (**Tab. 2**). This finding is consistent with Xia et al. [9], who demonstrated that the TSS levels of sea buckthorn juice do not change after 5 minutes of HPP at 500 MPa.

The TSS value slightly decreased during storage, with the control sample having the lowest value, which could be due to the continuous utilization of the sugars, particularly by psychotropic microorganisms during the storage period, as evidenced by a high viable cell count in the control sample. This finding is consistent with the fermented cashew apple juice, which exhibited a decrease in both monosaccharides and disaccharides after 42 days of refrigerated storage. This was linked to sugar fermentation by *L. casei* [11]. Furthermore, the decrease in TSS, particularly in samples with low microbial populations, could be attributed to acid-catalyzed sugar breakdown, which results in the synthesis of different intermediates that, in turn, induce a decrease in TSS.

The L* and a* values of the tepache were not significantly altered by HPP. However, an increase in the b* value was observed after HPP treatment (Day 0) (**Fig. 1**), indicating an increase in the beverage's yellowness. This result is consistent with the Chroma value, which improved with increasing HPP pressure, with HP600 displaying the most vivid and intense color, implying that HPP improves the color of the tepache. With the exception of the control, a slight decrease in L*, b*, and Chroma was observed after storage. This is consistent with the increase in a*, which could be due to non-enzymatic browning events that take place during storage. This finding could be explained by the fact that the HPP-treated stored samples had higher total soluble solids, which promote a greater degree of Maillard reactions. In addition, the decrease in the beverage brightness could be ascribed to the remaining polyphenol oxidase and peroxidase enzymes that survived the HPP treatments. These enzymes are associated with the degradation of phenolic compounds, leading to an accumulation of brown polymers [13].

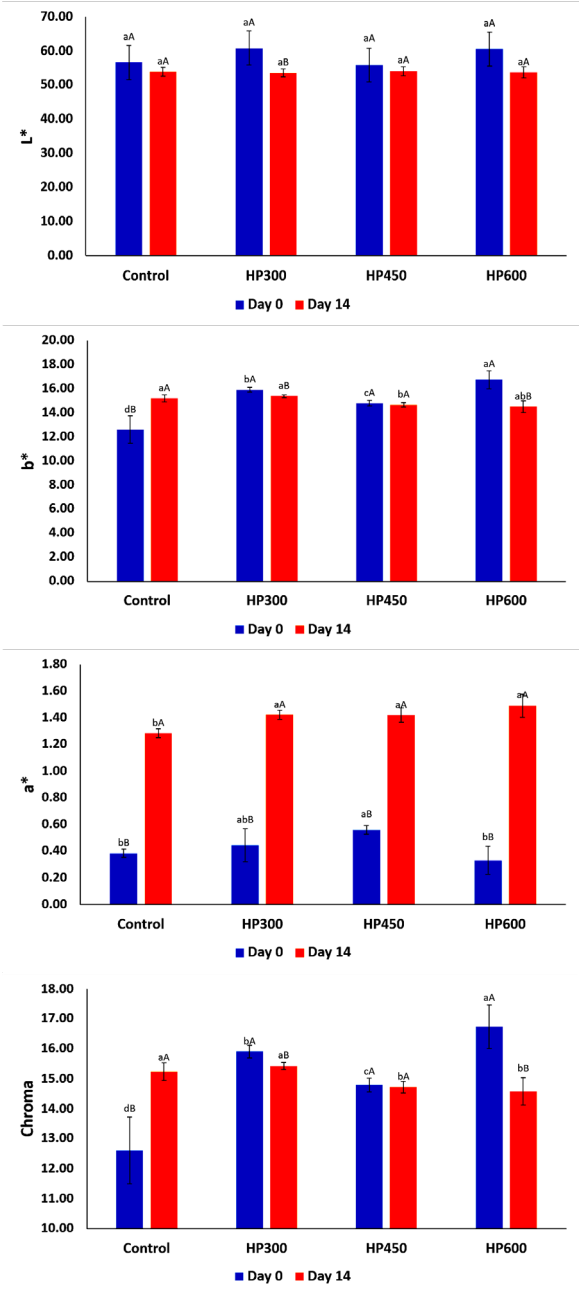

Fig. 1. Changes in color attributes of tepache following HPP treatment and storage.

The tepache's antioxidant activity remained stable throughout HPP treatment (Day 0), with DPPH scavenging activity ranging from 49.6 to 54.0%. The percentage of DDPH inhibition was slightly reduced during storage. The control sample had the lowest value, whereas antioxidant activity rose with increasing HPP pressure, with the stored HP600 sample exhibiting the highest value. The ascorbic acid equivalent (AAE) of the tepache ranged from 145.1 to 156.1 µg/mL. The HPP-treated samples showed a considerable improvement in AAE, which improved with increasing treatment pressure. Vitamin C breakdown during storage could account for the decrease in antioxidant efficacy. Previous authors [14] reported that

microorganisms can oxidize ascorbic acid to L-dehydroascorbic acid, which is then converted to L-ketogluconate, which lacks antioxidant properties and may explain the lower DPPH scavenging activity in non-HPP-treated samples.

Table 3. DPPH radical scavenging activity and ascorbic acid equivalent (AAE) of tepache following high-pressure processing (Day 0) and low-temperature storage (Day 14).

Samples	DPPH inhibition (%)		Ascorbic acid equivalent (µg/mL)	
	Day 0	Day 14	Day 0	Day 14
Control	51.6 ± 1.6^{aA}	49. ± 2.14^{aA}	152.7 ± 5.1^{aA}	145.1 ± 6.7^{aA}
HP300	52.8 ± 1.9^{aA}	51.2 ± 2.0^{aA}	156.5 ± 5.8^{aA}	151.5 ± 6.3^{aA}
HP450	52.4 ± 2.3^{aA}	51.6 ± 2.1^{aA}	155.3 ± 7.0^{aA}	153.0 ± 5.9^{aA}
HP600	54.0 ± 2.2^{aA}	52.6 ± 2.1^{aA}	160.3 ± 6.9^{aA}	156.1 ± 6.6^{aA}

Note: Values with different lowercase within the same column indicate significant differences across different samples ($p \leq 0.05$). Different uppercase letters within the same row indicate significant differences across storage durations for the same sample at $p \leq 0.05$.

The sensory evaluation of the tepache was performed on Day 0 of the samples. High-pressure treated samples scored higher for sweetness, sourness, aftertaste, and overall acceptability, whereas tepache treated at higher pressure (HP450 and HP600) had comparable scores (**Fig. 2**). The HPP-treated samples were perceived to have a better balance of sweetness and sourness, possibly due to lower acidity and higher total soluble sugars than control samples. Higher results for aftertaste imply that HPP treatment was able to reduce the undesirable aftertaste of the beverage. Color scores for all samples, including the control, ranged from 6.34±0.43 to 6.57±0.43, suggesting no noticeable difference between treated and untreated samples. In terms of aroma, all samples showed comparable scores, implying that the HPP was able to retain the volatile compounds in the beverage. The HP450 and HP600 had the highest overall acceptability, scoring 6.80±0.28 and 6.93±0.28, respectively, due to their balanced taste qualities.

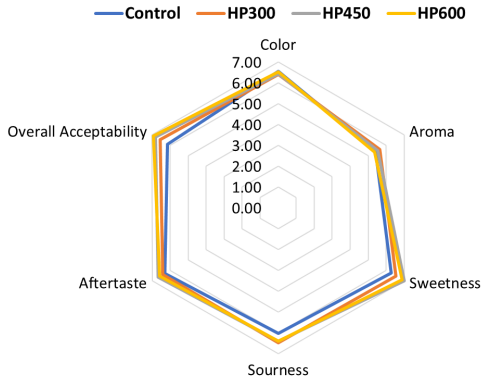

Fig. 2. Sensory attributes of tepache measured after high-pressure processing.

CONCLUSION

The high-pressure treatment led to a significant reduction in the microbial levels in the tepache while also improving the sensory quality of the beverage. Tepache treated with HPP exhibited a higher pH and total soluble solids, while maintaining color during storage. Although HPP treatment is unable to inactivate the microbial spores, the number of viable microorganisms is low after 14 days of storage, particularly when treated at 600 MPa. HPP treatment improved DPPH radical scavenging activity in tepache, with treatment at 600 MPa yielding the greatest ascorbic acid equivalent value. These findings support the use of HPP as a non-thermal option for improving the quality and shelf life of fermented fruit beverages while preserving their physicochemical and sensory properties.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

REFERENCES

1. Alvarado C, Garcia B, Martin S, Regalado C. Food-associated lactic acid bacteria with antimicrobial potential from traditional Mexican foods. Rev. Latinoam. Microbiol. 2006; 48: 260-268.
2. Gutiérrez-Sarmiento W, Peña-Ocaña BA, Lam-Gutiérrez A, Guzmán-Albores JM, Jasso-Chávez R, Ruíz-Valdiviezo VM. Microbial community structure, physicochemical characteristics and predictive functionalities of the Mexican tepache fermented beverage, Microbiol. Res. 2022; 260: 127045. https://doi.org/10.1016/j.micres.2022.127045
3. Corona-González RI, Ramos-Ibarra JR, Gutiérrez-González P, Pelayo-Ortiz C, Guatemala-Morales GM, Arriola-Guevara E. The use of response surface methodology to evaluate the fermentation conditions in the production of tepache. Rev. Mex. Ing. Quím. 2013; 12(1): 19-28.
4. de la Fuente-Salcido NM, Castañeda-Ramírez JC, García-Almendárez BE, Bideshi DK, Salcedo-Hernández R, Barboza-Corona, JE. (2015). Isolation and characterization of bacteriocinogenic lactic bacteria from M-Tuba and Tepache, two traditional fermented beverages in México. Food Sci. Nut. 2015; 3(5): 434–442. https://doi.org/10.1002/fsn3.236
5. Romero-Luna HE, Peredo-Lovillo A, Dávila-Ortiz G. 2022. Tepache: a pre-hispanic fermented beverage as a potential source of probiotic yeasts. Hispanic foods: Chemistry of fermented foods, Chap. 9, In ACS Symposium SeriesVol. 1406. Ilinois: American Chemical Society; 2022: 135-147. https:doi//.org/10.1021/bk-2022-1406.ch009
6. Ma Y, Yin X, Bi X, Su F, Liang Z, Luo M, Fu D, Xing Y, Che Z. (2019). Physicochemical properties and bioactive compounds of fermented pomegranate juice as affected by high-pressure processing and thermal treatment. Int. J. Food Prop. 2019; 22(1): 1250–1269. https://doi.org/10.1080/10942912.2019.1640737
7. Arifan F, Winarni S, Wahyuningsih, Pudjihastuti I, Wisnu Broto RTD. Total plate count (TPC) analysis of processed ginger on tlogowungu tourism village. Adv. Eng. Res. 2018; 167: 377-379. https://doi.org/10.2991/icoma-18.2019.80
8. Choi MH, Kim GH, Lee HS. Effects of ascorbic acid retention on juice color and pigment stability in blood orange (*Citrus sinensis*) juice during refrigerated storage. Food Res. Int. 2002; 35(8): 753-759. https://doi.org/10.1016/S0963-9969(02)00071-6
9. Oikeh EI, Omoregie ES, Oviasogie FE, Oriakhi K. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. Food Sci. Nut. 2016; 4(1): 103–109. https://doi.org/10.1002/fsn3.268
10. Xia Q, Liu Q, Cao Y, Zhao Y, Lu S, Wu D, Guan AR. Improving quality of sea buckthorn juice by high-pressure processing. LWT. 2023;185: 115149. https://doi.org/10.1016/j.lwt.2023.115149
11. Pereira ALF, Almeida FDL, de Jesus ALT. Storage stability and acceptance of probiotic beverage from cashew apple juice. Food Bioprocess Technol. 2013; 6: 3155–3165. https://doi.org/10.1007/s11947-012-1032-1
12. Abdurrahman FHR, Titik S. Antioxidant activity and organoleptic quality of probiotic tepache of pineapple peel sugar variation and fermentation duration. Proceeding of International Conference on Biology Education, Natural Science, and Technology (INCOBEST), 2023; 1(1): 502-508.
12. Truffault V, Fry SC, Stevens RG, Gautier H. Ascorbate degradation in tomato leads to accumulation of oxalate, threonate and oxalyl threonate. Plant J. 2017; 89: 996–1008. https://doi.org/10.1111/tpj.13439
13. Nokthai P, Lee VS, Shank L. Molecular modeling of peroxidase and polyphenol oxidase: substrate specificity and active site comparison. Int. J. Mol. Sci. 2010; 11: 3266–3276. https://doi.org/10.3390/ijms11093266
14. Sutedjo KSD, Nisa FC. Star fruit (*Averrhoa carambola L.*) concentrate and fermentation period in physic-chemical microbiology properties of yoghurt. J. Pangan Agroindustri 2015; 3(2): 582–593