JBCT Journal of BioProcess and Chemical Technology
2025 Volumen 17, No. 35
Revista Científica de la Universidad Autónoma de Coahuila

Increased Antioxidant Activity of *Flourensia cernua* by Fermentation with Kombucha

Incremento de la actividad antioxidante de *Flourensia cernua* mediante Fermentación con Kombucha

Rodríguez-González, J.G.[3], Ascacio Valdés, J.A.[2], Meléndez Rentería, N.P.[4], Ríos-Saldaña, C.A.[3], Rodríguez-Martínez, L.M.[3], Laredo Alcalá, E.I.[1], De León-Zapata, M.A.[1*]

[1]*Centro de Investigación para la Conservación de la Biodiversidad y Ecología de Coahuila, Universidad Autónoma de Coahuila, 27540, Cuatro Ciénegas, Coahuila, México.*
[2]*Departamento de Investigación en Alimentos, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, 25280, Saltillo, Coahuila, México.*
[3]*Centro de Estudios e Investigaciones Interdisciplinarios, Universidad Autónoma de Coahuila, 25350, Saltillo, Coahuila, México.*
[4]*Centro de Investigación e Innovación Científica y Tecnológica, Universidad Autónoma de Coahuila, 25070, Saltillo, Coahuila, México.*

**Corresponding Author: miguel.leon@uadec.edu.mx*

Recibido: 07 de febrero de 2025
Aceptado: 17 de junio de 2025

Resumen

Flourensia cernua es conocida como hojasén y se utiliza para tratar problemas gastrointestinales por su contenido de compuestos fenólicos. Estos compuestos son estructuras químicas antioxidantes que se encuentran polimerizadas en tejidos vegetales, por lo que es necesaria su hidrólisis mediante bioprocesos sustentables como la fermentación. El kombucha es un cultivo simbiótico de varias bacterias y levaduras, capaces de producir enzimas para la liberación de los taninos. El presente estudio se enfocó en incrementar la actividad antioxidante de *F. cernua* mediante un proceso de fermentación de kombucha durante 2 semanas a temperatura ambiente. Por lo que se obtuvieron infusiones acuosas fermentadas y no fermentadas (control) para medir su composición química y antioxidante. La infusión de *F. cernua* fermentada con kombucha aumentó significativamente el contenido de taninos totales y el potencial antioxidante. La actividad antioxidante se atribuye principalmente a flavononas, lignanos, ácidos hidroxicinámicos y flavonoides, identificados mediante HPLC-MS en infusiones fermentadas de hojas de *F. cernua*. Los resultados obtenidos sugirieron que la fermentación es un bioproceso prometedor, simple y seguro que podría mejorar las propiedades biológicas de las plantas comestibles menos utilizadas como el hojasén *F. cernua*.

Palabras clave: Antioxidante, bioproceso, Cuatro Ciénegas, *Flourensia cernua*, sustentabilidad.

Abstract

Flourensia cernua, known as tarbush, treats gastrointestinal problems due to its phenolic compound content. These compounds are antioxidant chemical structures found polymerized in plant tissues, requiring their hydrolysis through sustainable bioprocesses such as fermentation. Kombucha is a symbiotic culture of several bacteria and yeasts, which can produce enzymes to release tannins. The present study focused on increasing the antioxidant activity of *F. cernua* through a kombucha fermentation process for 2 weeks at room temperature. Obtained fermented and non-fermented aqueous infusions (control) to measure their chemical and antioxidant composition. Infusion of *F. cernua* fermented with kombucha significantly increased the total tannin content and antioxidant potential. Antioxidant activity attributed to flavanones, lignans, hydroxycinnamic acids, and flavonoids identified by HPLC-MS in fermented infusions of *F. cernua*. The results suggested that fermentation is a promising, safe, and straightforward bioprocess that could improve the biological properties of less-used edible plants such as *F. cernua*.

Keywords: Antioxidant, bioprocess, Cuatro Ciénegas, *Flourensia cernua*, sustainability.

INTRODUCTION

Flourensia cernua is a perennial shrub that grows in semiarid areas. Found in the deserts of Chihuahua and Sonora, where it has commonly been used in traditional medicine for the treatment of stomach pain, diarrhea, dysentery, purgative (Jasso de Rodríguez et al., 2019), antirheumatic, venereal diseases, herpes, bronchitis, chickenpox, and common cold (Ventura et al., 2009).

In addition, leaf extracts of *F. cernua* reported to have antioxidant and antifungal (Jasso de Rodríguez et al., 2011; De León-Zapata et al., 2013; De León-Zapata et al., 2016; Jasso de Rodríguez et al., 2017), insecticidal (Téllez et al., 2001), antibacterial (Méndez et al., 2012) and antitumor (MacRae & Towers, 1984) properties.

The biological activity of *F. cernua* is due to its chemical composition mainly by phenolic compounds such as long-chain hydrocarbons, lactones (Jasso De Rodríguez et al., 2007), saponins (Méndez et al., 2012), terpenes (Estell et al., 2013), condensed tannins, (De León Zapata et al., 2013) and flavonoids (Álvarez-Pérez et al., 2020).

Currently, for the extraction of phenolic compounds from *F. cernua*, solvents such as water, methanol, ethanol, hexane, chloroform and diethyl ether have been used; in addition to a solid fermentation process of *F. cernua* leaves with filamentous fungi (Guerrero et al., 2007; Jasso-De Rodríguez et al., 2007; De León Zapata et al., 2013; De León Zapata et al., 2016). However, *F. cernua* is a non-timber forest resource with great biotechnological potential that is poverly exploited in northern Mexico's arid zones.

On the other hand, kombucha is a beverage obtained by fermentation with a symbiotic culture of several indigenous bacteria (*Acetobacter* and *Gluconobacter*) and yeasts (*Saccharomyces* spp and non-*Saccharomyces* spp) (Malbasa et al., 2015). Most studies suggest that "kombucha" comes from Southeast Asia, Japan, Tibet, or Manchuria and dates back thousands of years (Jarrell et al., 2000). An undulating cellulose film and an acidic liquid broth are the two portions that form "kombucha." Furthermore, kombucha drinks have been claimed to be a prophylactic agent beneficial to health (Villarreal-Soto et al., 2018).

Nowadays, kombucha preparation is not limited to sweetened black tea. Substrates, such as fruit drinks, wine, milk, herbal teas, lemon balm, and green tea, can be used instead of tea. Some new substrates stimulate kombucha fermentation better than the original kombucha tea (Vitas et al., 2013).

Therefore, in the present study, *F. cernua* plant material was subjected to liquid fermentation with kombucha to increase its antioxidant properties.

MATERIALS AND METHODS

Material and reagents

The leaves of *F. cernua* were collected in the rural community of La Vega, Cuatro Ciénegas, Coahuila, Mexico, belonging to Ramsar site number 734 of Cuatro Ciénegas, recognized for its high biodiversity and endemism. The plant material was dehydrated for 10 days, pulverized, and stored in plastic bags at room temperature (25 °C) until use. Moreover, the reagents that correspond to 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin Ciocalteu reagent, gallic acid, ethanol, acetonitrile, acetic acid, sodium carbonate, HCl, $K_2S_2O_8$, KH_2PO_4, MeOH, NaOH y Na_2HPO_4, were purchased from Sigma Aldrich.

Obtaining the kombucha culture and inoculum preparation

Traditional kombucha was cultivated in the laboratory of the Food Research Department of the Faculty of Chemical Sciences of the Autonomous University of Coahuila (Mexico) and used as the initial starter for fermentation. The inoculum was according to the methodology described by Jayabalan et al. (2014), with adaptations. Dried leaves of *F. cernua* were used as raw material for the infusion. Sucrose (50 g) was dissolved in 1000 mL of purified water and heated to 90 °C. Then, 12 g of leaf powder was introduced into a small muslin cloth bag and placed in boiling water for 5 minutes. Afterward, the preparation was allowed to cool at 30 °C before adding 7 g of the kombucha strain. The kombucha strain was cultured in the previously cooled medium for 14 days at a temperature of 25 °C.

To prepare the unfermented infusion as a control, 12 g of leaf powder was introduced into a small muslin cloth

bag, which was placed in 1000 mL of purified water at 90 °C for 5 minutes. Afterward, the aqueous infusion was allowed to cool to 30 °C, stored in a previously disinfected amber plastic container, and frozen at -5 °C until use.

Determination of pH and TSS

The total soluble solids (TSS) content of the fermented and unfermented samples was determined by refractometry using a digital refractometer (Atago Co., Tokyo, Japan) with automatic temperature compensation. The results are in % (w/w sucrose concentration). The pH values of the fermented and unfermented samples were measured using an electronic pH meter (pH2700, Eutech, Thermo Fisher Scientific, USA).

Total yeast count

Fermented samples were taken at 3, 7, 10, and 14 days of fermentation. After gently mixing the fermented brew, 1 mL samples were placed in Eppendorf tubes. A Thoma cell counting chamber (Thomas Scientific, Swedesboro, NJ, USA) was used to count yeast. For microscopic observations, one drop of fermented brew was placed into a coverslip and observed on the stage of a binocular microscope (Laborlux 12 microscope, Leitz, Midland, ONT, Canada) for further observation at 10 or 40x magnification.

Determination of antioxidant activity

DPPH assay performed as described by De León-Zapata et al. (2021). The cation radical ABTS was synthesized by a 7 mM ABTS solution reaction with a 2.45 mM $K_2S_2O_8$ solution. The mixture was kept at 23 ± 1 ºC in the dark for 16 h. Afterward, the ABTS solution was diluted with ethanol until a UV- Vis spectrophotometer achieved an absorbance of 0.7 at 734 nm. 10 µL of the sample (fermented and non-fermented infusion) was added in the reaction cuvette immediately after 1 mL of ABTS solution was added. After 10 min, the percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to the blank absorbance (ethanol).

The DPPH radical is characterized by an unpaired electron, a free radical stabilized by resonance. A solution of DPPH radical at a concentration of 60 mM by diluting with methanol was prepared. 100 µL of the sample (fermented and non-fermented infusion) was added in test tubes covered with foil, plus 2.9 mL of DPPH solution, and allowed to stand for 30 min. The absorbance was recorded at a wavelength of 517 nm.

The results were expressed in DPPH and ABTS radicals percentage of inhibition (%).

Total tannins

Total tannins were determined according to the methodology described by Wong-Paz et al. (2014) using the Folin-Ciocalteu reagent. In a microplate well, 20 µL of sample was added. Subsequently, 20 µL of Folin-Ciocalteu's reagent was added and mixed for 5 min. Subsequently, 20 µL of sodium carbonate (10 mM) was added to the mixture and allowed to react for another 5 min. Finally, 125 µL of distilled water was added, and the absorbance was read at 790 nm using a microplate reader. The results were expressed as milligrams of gallic acid equivalents (GAE) per milliliter of fermented and non-fermented infusion (mg GAE/mL) according to a gallic acid standard curve (0-1,000 mg/L; $R^2 = 0.997$).

High-performance liquid chromatography-mass spectrometry assay (HPLC-MS)

Samples of fermented and non-fermented infusions were filtered through a nylon membrane (pore size 0.45 µm), 1.5 mL of infusion was placed in 1.8 mL capacity vials for HPLC analysis (Alliance HPLC, Water e2695) with a UV-vis photodiode array detector at 280 nm. The determination was performed under the following operating conditions: Denali C18 column, mobile phase A was ethanol (wash phase), phase B was acetonitrile, and phase C was 3 % acetic acid in gradient elution, and the injection volume was 10 µl. A Varian 500/MS mass spectrometry with a flow rate of 1 mL/minute was used, and the detection of the mass range was 100-2000 for 10 minutes (Ascacio-Valdés et al., 2010).

Statistical analysis

Results were evaluated using ANOVA with six replicates. Values reported are the average of measurements and were compared using the Tukey multiple range test with a significance level of $p < 0.05$. Data analysis was conducted using the Statistica 7.0. Pearson's correlation was calculated using Microsoft Excel.

RESULTS AND DISCUSSIONS

Total yeast count and determination of pH and TSS

The results from the total yeast count indicated that after 10 days of incubation, the total yeast count increased slightly from 1.6E+07 to 1.7E+07 cells/mL in the liquid (Table 1). At the end of fermentation, yeast growth remained constant at 1.7E+07 cells/mL (Table 1).

Table 1. Effect of the fermentation of infusions of *F. cernua* with kombucha on yeast growth, pH, and total soluble solids (TSS).

Fermentation time (Days)	Yeast count (log CFU/mL)	pH	TSS (%)
0	0^{e}	7.0^{e}	5.4^{e}
3	$1.00E+06^{d}$	6.3^{d}	5.1^{d}
7	$1.30E+07^{c}$	5.7^{c}	4.9^{c}
10	$1.60E+07^{b}$	5.4^{b}	4.4^{b}
14	$1.70E+07^{a}$	5.0^{a}	4.1^{a}

Within each column, different letters represent a significant difference ($P < 0.05$). CFU: Colony Forming Units.

The pH values of the fermented *F. cernua* leaf infusion had a significant decrease ($p \leq 0.05$) compared to the non-fermented control (pH = 7). A rapid decrease in the pH of the infusion was observed during the first week, falling to pH = 5 at the end of fermentation (Table 1).

Furthermore, the total soluble solids (TSS) values decreased significantly ($p \leq 0.05$) from 5.4% to 4.1% as the fermentation process proceeded (Table 1). The role of yeasts in kombucha fermentation is important because they are known to be responsible for ethanol production through the hydrolysis of sucrose (Watawana et al., 2015; Sievers et al., 1995). The behavior observed in the present study, where sucrose was consumed by kombucha yeasts (Table 1). Moreover, acetic acid bacteria use ethanol to produce acetic acid, the main fermentation product of kombucha and the main reason for decreased pH (Jayabalan et al., 2014) (Table 1). The results were consistent with previous work reporting acetic acid and ethanol content changes during kombucha fermentation (Abbott & Ingledew, 2004; Sievers et al., 1995; Yang et al., 2016). The pH changes obtained are similar to those found by Velicanski et al. (2013), who used *Lamiaceae* as a substrate for kombucha fermentation in small bioreactors. Several investigations have reported that low pH has many beneficial effects, such as protecting the bioactivity of phenolic compounds and the safety of fermented infusions against pathogenic microorganisms (Lucera et al., 2012).

Antioxidant activity and total tannins

Figures 1 and 2 show the antioxidant activity values measured as the capacity of the infusion to reduce DPPH and ABTS free radicals, respectively.

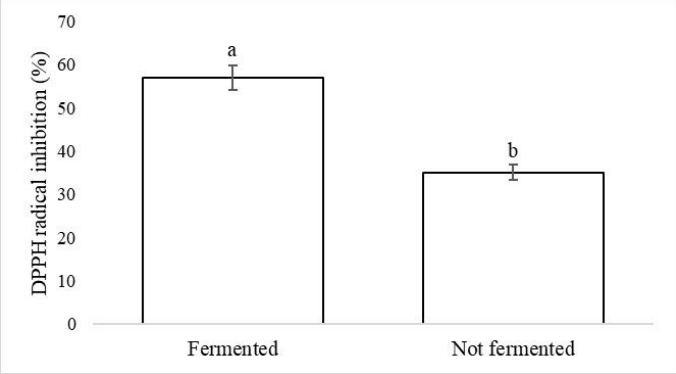

Figure 1. Antioxidant activity of fermented and unfermented *F. cernua* infusions by DPPH assay. Different letters indicate significant differences at $p < 0.05$.

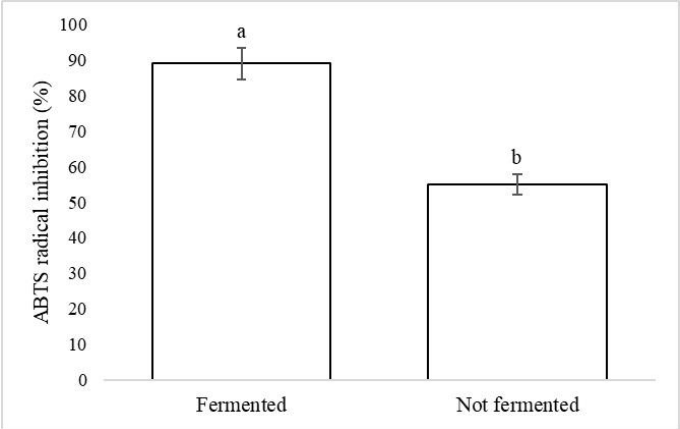

Figure 2. Antioxidant activity of fermented and unfermented *F. cernua* infusions by ABTS assay. Different letters indicate significant differences at $p < 0.05$.

There was a significant increase ($p \leq 0.05$) in the antioxidant activity by DPPH (Figure 1) and ABTS (Figure 2) of the fermented samples compared to the non-fermented ones. The fermented samples showed a higher antioxidant power for scavenging ABTS radicals (Figure 2) than the DPPH radical (Figure 1). The obtained values of antioxidant activity of the fermented samples by ABTS and DPPH were 89% and 57%, respectively, compared to the non-fermented samples (33%).

Figure 3 shows a significant increase ($p \leq 0.05$) can be observed in the total tannin content of the fermented samples (1152 mg gallic acid equivalents/mL) compared to the unfermented samples (109 mg of gallic acid equivalents/mL). The total tannin content of the unfermented samples (109 mg gallic acid equivalents/mL) (Figure 3) was higher than that reported by Méndez et al. (2012) in an aqueous extract of *F. cernua* (4.76 of gallic acid equivalents/g) because the extract was subjected to 60°C for a longer time (7 hours) than in the present study (10 minutes at 90°C), which may accelerate the degradation of phenolic compounds.

The high values of total tannins in the fermented samples (Figure 3) are much higher than those reported by other authors in extracts of *F. cernua* using water, ethanol, hexane and ether, methanol-chloroform as extraction solvents (De León-Zapata et al., 2016; Álvarez-Pérez et al., 2020; Guerrero et al., 2007).

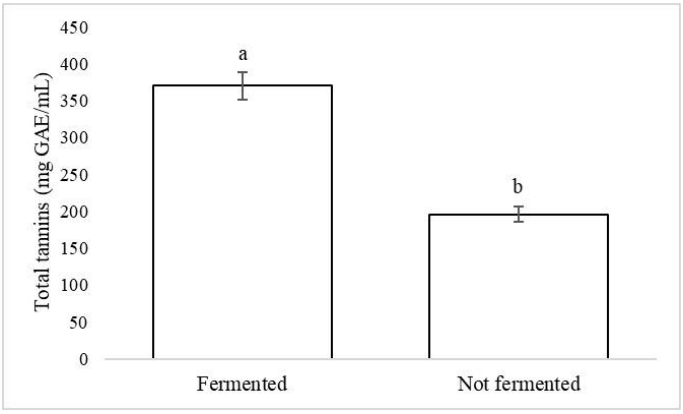

Figure 3. The total tannin content of fermented and unfermented *F. cernua* infusions. Different letters indicate significant differences at $p < 0.05$.

This behavior relates to the biotransformation of secondary metabolites present in *F. cernua* by yeasts, bacteria, and fungi in kombucha (Watawana et al., 2015). Some studies have corroborated this behavior by demonstrating that fermentation is a bioprocess that allows obtaining tannin-rich beverages from black tea, sweetened lemon balm, and different fruits and vegetables (Velicanski et al., 2013; Yang et al., 2016). Fermented samples of *F. cernua* showed a higher antioxidant power for scavenging ABTS radicals (Figure 2) than DPPH radicals (Figure 1); this is due to the sensitivity of the ABTS radical as it is a structure that easily reacts with hydrophilic and lipophilic compounds (Jasso de Rodríguez et al., 2023), and reducing agents (De León-Zapata et al., 2016) such as total and reducing sugars present in tarbush (Belmares et al., 2009). On the other hand, the DPPH radical reacts with hydrophilic compounds such as gallic acid (Álvarez et al., 2020). The antioxidant activity is higher than that reported in an aqueous extract of fermented tarbush in a solid medium (De León-Zapata et al., 2013). The amount of total tannins and the radical scavenging activity of DPPH and ABTS showed a concentration-dependent relationship. This is consistent with the fact that the antioxidant activity of phenolic compounds is mainly due to the number of hydroxyl groups, as well as their redox properties (De León-Zapata et al., 2013).

Tannins are highly soluble due to the interaction of water with hydroxyl groups and carboxylic acids (Méndez

et al., 2012). A high content of phenolic compounds could initially suggest a good antioxidant capacity (Cheung et al., 2003). The results show that at the highest concentration of total tannins (Figure 3) in the fermented samples, the highest values of antioxidant activity by DPPH (Figure 1) and ABTS (Figure 2) were obtained due to the greater amount of available hydroxyl groups. Flavonoids and hydrolyzable tannins contain in their chemical structure a variable number of hydroxyl groups (Jasso de Rodríguez et al., 2023), which are involved in the neutralization of free radicals by electron donation and, therefore, influence the antioxidant activity (De León-Zapata et al., 2013).

High-performance liquid chromatography-mass spectrometry assay (HPLC-MS)

Figure 4 shows the chromatographic profile of the main phenolic compounds of unfermented *F. cernua* samples at different retention times where the main signals correspond to (A) Caffeic acid 4-O-glucoside (Hydroxycinnamic acids), (B) Syringaresinol (Lignans), (C) Phloretin (Dihydrochalcones), (D) Caffeoyl tartaric acid (Hydroxycinnamic acids) and (E) Apigenin galactoside-arabinoside (Flavonoid).

Furthermore, Figure 5 shows the chromatographic profile of the main phenolic compounds of the fermented samples of *F. cernua* at different retention times where the main signals correspond to (A) Pinocembrin (Flavanones), (B) Syringaresinol (Lignans), (C) 4-Caffeoylquinic acid (Hydroxycinnamic acids), (D) Caffeoyl tartaric acid (Hydroxycinnamic acids) and (E) Apigenin galactoside-arabinoside (Flavonoid), not yet reported in extracts of *F. cernua*.

These results are similar to those reported by De León-Zapata et al. (2016) and Álvarez-Pérez et al. (2020), who reported the identification of flavonoids such as luteolin 7-O-rutinoside, 6-C-glucosyl-8-C-arabinosyl apigenin and apigenin galactoside arabinoside in resins obtained from an aqueous extract of *F. cernua*, and by Aranda-Ledesma et al. (2022), who identified apigenin-6-C-glucosyl-8-C-arabinoside by UPLC/QToF-MS2 in *F. cernua* essential oil.

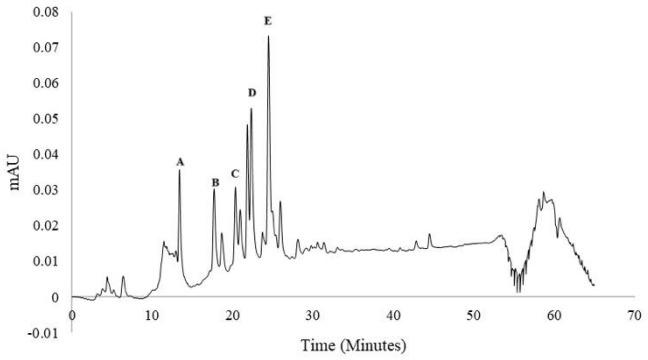

Figure 4. Chromatographic profile of the main phenolic compounds recorded in unfermented samples of *F. cernua* at different retention times. (A) Caffeic acid 4-O-glucoside, (B) Syringaresinol, (C) Phloretin, (D) Caffeoyl tartaric acid y (E) Apigenin galactoside-arabinoside.

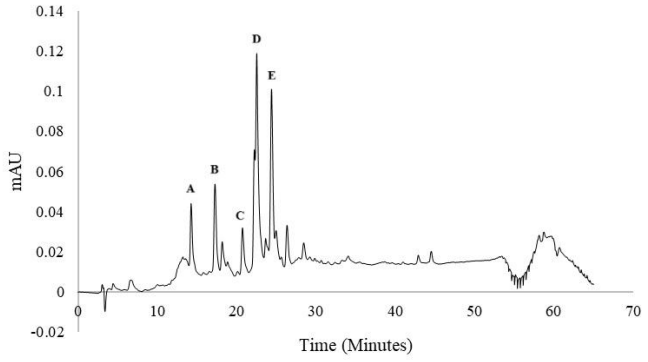

Figure 5. Chromatographic profile of the main phenolic compounds recorded in fermented samples of *F. cernua* at different retention times. (A) Pinocembrin, (B) Syringaresinol, (C) 4-Caffeoylquinic acid, (D) Caffeoyl tartaric acid y (E) Apigenin galactoside-arabinoside.

The presence of a higher amount of phenolic compounds, as in the fermented samples of *F. cernua* (Figure 5), suggests a higher amount of tannins and, therefore, a higher amount of hydroxyl groups (Jasso-De Rodríguez et al., 2007), which are responsible for the antioxidant activity by neutralizing free radicals. These natural phenolic compounds are flavonoids found in foods of plant origin (Scalbert & Williamson, 2000). Tarbush *F. cernua* plant uses these natural chemical compounds as resistance against microorganisms, rodents, insects, etc. (Belmares et al., 2009; Méndez et al., 2012). Fermented

infusions of *F. cernua* represent a rich natural source of phenolic compounds with antioxidant activity; this is more so when compounds are extracted from natural and organic hydrolytic bioprocesses such as fermentation with kombucha (Rahmani et al., 2019).

CONCLUSIONS

Phytochemical analysis of fermented and unfermented *F. cernua* infusions showed that fermentation increased the total tannin content and enhanced antioxidant activity. The present results are the first report to identify flavanones, lignans, hydroxycinnamic acids, and flavonoids by HPLC-MS of fermented *F. cernua* leaves infusions obtained by a bioprocess of fermentation with kombucha. *F. cernua* leaf resulted in a promising natural source for the recovery of high-added value compounds with antioxidant activity and, at the same time, increased the added value of this plant widely distributed in semiarid regions of Mexico. Its controlled use only involves harvesting or pruning the upper third of the foliage and would adhere to the Forestry Law on non-timber forest resources. The fermented *F. cernua* leaves infusion with kombucha represents a natural alternative with antioxidant properties, which gives it great therapeutic potential against oxidative processes at the cellular level for the treatment of chronic and degenerative diseases, such as cancer, arthritis, neurodegenerative diseases, and cardiovascular diseases.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Public Education for its financial support through the Higher Education Teacher Professional Development Program Fund for incorporating NPTC and the Autonomous University of Coahuila.

REFERENCES

Abbott DA and Ingledew WM. 2004. The buffering capacity of whole corn mash alters concentrations of organic acids required to inhibit the growth of *Saccharomyces cerevisiae* and ethanol production. Biotechnology Letters, 26(16): 1313-1316. https://doi.org/10.1023/b:bile.0000044924.76429.71

Álvarez-Pérez OB, Ventura-Sobrevilla JM, Ascacio-Valdés JA, Rojas R, Verma DK and Aguilar CN. 2020. Valorization of *Flourensia cernua* DC as source of antioxidants and antifungal bioactives. Industrial Crops and Products, 152: 112422. https://doi.org/10.1016/j.indcrop.2020.112422

Aranda-Ledesma NE, González-Hernández MD, Rojas R, Paz-González AD, Rivera G, Luna-Sosa B, Martínez-Ávila GCG. 2022. Essential Oil and Polyphenolic Compounds of Flourensia cernua Leaves: Chemical Profiling and Functional Properties. Agronomy, 12: 2274. https://doi.org/10.3390/agronomy12102274

Ascacio-Valdés JA, Aguilera-Carbó AF, Martínez-Hernández JL, Rodríguez-Herrera R and Aguilar CN. 2010. *Euphorbia antisyphilitica* residues as a new source of ellagic acid. Chemical Papers, 64: 528-532. https://doi.org/10.2478/s11696-010-0034-6

Belmares R, Garza Y, Rodríguez R, Contreras-Esquivel JC and Aguilar CN. 2009. Composition and fungal degradation of tannins present in semiarid plants. Electronic Journal of Environmental, Agricultural and Food Chemistry, 8: 312-318.

Cheung LM, Cheung HPCK and Ooi VEC. 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chemistry, 81: 249-255. https://doi.org/10.1016/S0308- 8146(02)00419-3

De León MA, Sáenz A, Jasso-Cantu D, Rodríguez R, Pandey A and Aguilar CN. 2013. Fermented *Flourensia cernua* extracts and their in vitro assay against *Penicillium expansum* and *Fusarium oxysporum.* Food Technology and Biotechnology, 51(2): 233-239.

De León-Zapata MA, Pastrana-Castro L, Rúa-Rodríguez ML, Álvarez-Pérez OB, Rodríguez-Herrera R and Aguilar CN. 2016. Experimental protocol for the recovery and evaluation of bioactive compounds of tarbush against postharvest fruit fungi. Food Chemistry, 198: 62-67. https://doi.org/10.1016/j.foodchem.2015.11.034

De León-Zapata M, Pastrana-Castro L, Barbosa-Pereira L, Rúa-Rodríguez ML, Ventura J, Salinas T, Rodríguez R and Aguilar CN. 2021. Effect of *Flourensia cernua* bioactive compounds on stability of an oil-in-water (O/W) emulsion. Biointerface Research in Applied Chemistry, 11(6): 13997-14006. http://dx.doi.org/10.33263/BRIAC116.1399714006

Estell RE, James DK, Fredrickson EL and Anderson DM. 2013. Within-plant distribution of volatile compounds on the leaf surface of *Flourensia cernua*. Biochemical Systematics and Ecology, 48: 144-150. https://doi.org/10.1016/j.bse.2012.11.020

Guerrero-Rodríguez E, Solis-Gaona S, Hernández-Castillo FD, Flores-Olivas A, Sandoval-López V and Jasso-Cantú D. 2007. In vitro biological activity of extracts from Flourensia cernua D.C. in post-harvest pathogens: Alternaria alternata (Fr.:Fr.) Keissl., Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. and Penicillium digitatum (Pers.:Fr.) Sacc., Rev. Mex. Phytopathol. 25: 48-53 (in Spanish).

Jarrell J, Cal T and Bennett JW. 2000. The Kombucha consortia of yeasts and bacteria. Mycologist. 14(4): 166-170. https://doi.org/10.1016/S0269-915X(00)80034-8

Jasso de Rodríguez D, Puente-Romero GN, Díaz-Jiménez L, Rodríguez-García R, Ramírez-Rodríguez H, Villarreal-Quintanilla JA, Flores-López ML, Carrillo Lomelí DA and Genisheva ZA. 2019. In vitro gastrointestinal digestion of microencapsulated extracts of *Flourensia cernua*, *F. microphylla*, and *F. Retinophylla*. Industrial Crops and Products, 138: 111444. https://doi.org/10.1016/j.indcrop.2019.06.007

Jasso de Rodríguez D, Salas-Méndez E de J, Rodríguez-García R, Hernández-Castillo FD, Díaz-Jiménez MLV, Sáenz-Galindo A, González-Morales S, Flores-López ML, Villarreal-Quintanilla JA, Peña-Ramos FM and Carrillo-Lomelí DA. 2017. In vitro antifungal activity of ethanol and aqueous extracts of leaves and branches of *Flourensia* spp. against postharvest fungi. Industrial Crops and Products, 107: 499-508. https://doi.org/10.1016/j.indcrop.2017.04.054

Jasso-De Rodríguez D, Hernández CD, Angulo SJL, Rodríguez GR, Villarreal QJA and Lira SRH. 2007. Antifungal activity in vitro of *F. cernua* extracts on *Alternaria* sp., *Rhizoctonia solani*, and *Fusarium oxysporum*. Industrial Crops and Products, 25, 111-116. http://dx.doi.org/10.1016/j.indcrop.2006.08.007

Jasso-De Rodríguez D, Rodríguez-García R, Hernández-Castillo FD, Aguilar-González CN, Sáenz-Galindo A, Villarreal-Quintanilla JA and Moreno-Zuccolotto LE. 2011. In vitro antifungal activity of extracts of Mexican Chihuahuan Desert plants against postharvest fruit fungi. Industrial Crops and Products, 34: 960-966. https://doi.org/10.1016/j.indcrop.2011.03.001

Jasso de Rodríguez D, Torres-Moreno H, López-Romero JC, Vidal-Gutiérrez M, Villarreal-Quintanilla JA, Carrillo-Lomelí DA, Robles-Zepeda RE and Vilegas W. 2023. Antioxidant, anti-inflammatory, and antiproliferative activities of Flourensia spp. Biocatalysis and Agricultural Biotechnology, 47: 102552. https://doi.org/10.1016/j.bcab.2022.102552

Jayabalan R, Malbasa RV, Lončar ES, Vitas JS and Sathishkumar M. 2014. A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and "tea fungus". Comprehensive Reviews in Food Science and Food Safety, 13(4): 538-550. http://dx.doi.org/10.1111/1541-4337.12073

Lucera A, Costa C, Conte A and Del-Nobile MA. 2012. Food applications of natural antimicrobial compounds. Frontiers in Microbiology, 3: 1-13. https://dx.doi.org/10.3389%2Ffmicb.2012.00287

MacRae WD and Towers GHN. 1984. Biological activities of lignans. Phytochemistry, 23: 1207-1220. https://doi.org/10.1016/S0031-9422(00)80428-8

Malbasa R, Jevrić L, Lončar E, Vitas J, Podunavac-Kuzmanović S, Milanović S and Kovačević S. 2015. Enfoque quimiométrico para el análisis del perfil de textura de los productos lácteos fermentados con kombucha. Journal of Food Science and Technology, 52: 5968-5974. https://doi.org/10.1007/s13197-014-1648-4

Méndez M, Rodríguez R, Ruiz J, Morales-Adame D, Hernández-Castillo FD and Aguilar CN. 2012. Antibacterial activity of plant extracts obtained with alternative organic solvents against food-borne pathogen bacteria. Industrial Crops and Products, 37: 445-450. http://dx.doi.org/10.1016/j.indcrop.2011.07.017

Rahmani R, Beaufort S, Villarreal-Soto SA, Taillandier P, Bouajila J and Debouba M. 2019. Kombucha fermentation of African mustard (*Brassica tournefortii*) leaves: Chemical composition and bioactivity. Food Bioscience, 30: 100414. https://doi.org/10.1016/j.fbio.2019.100414.

Scalbert A and Williamson G. 2000. Dietary intake and bioavailability of polyphenols. The Journal of

Nutrition, 130: 2073-2085. https://doi.org/10.1093/jn/130.8.2073s

Sievers M, Lanini C, Weber A, Schuler-Schmid U and Teuber M. 1995. Microbiology and fermentation balance in a kombucha beverage obtained from a tea fungus fermentation. Systematic and Applied Microbiology, 18(4): 590-594. https://doi.org/10.1016/S0723-2020(11)80420-0

Téllez M, Estell R, Fredrickson E, Powell J, Wedge D, Schrader K and Kobaisy M. 2001. Extracts of *Flourensia cernua* (L): volatile constituents and antifungal, antialgal, and antitermite bioactivities. Journal of Chemical Ecology, 27: 2263-2273.

Velicanski A, Cvetkovic D and Markov S. 2013. Characteristics of kombucha fermentation on medicinal herbs from Lamiaceae family. Romanian Biotechnological Letters, 18(1): 8034-8042.

Ventura J, Gutiérrez-Sánchez G, Rodríguez-Herrera R y Aguilar CN. 2009. Fungal cultures of tar bush and creosote bush for production of two phenolic antioxidants (pyrocatechol and gallic acid). Folia Microbiol (Praha). 54(3): 199-203. https://doi.org/10.1007/s12223-009-0031-8

Villarreal-Soto SA, Beaufort S, Bouajila J, Souchard JP and Taillandier P. 2018. Understanding kombucha tea fermentation: A review. Journal of Food Science, 83(3): 580-588. https://doi.org/10.1111/1750-3841.14068

Vitas JS, Malbasa RV, Grahovac JA and Lončar ES. 2013. The antioxidant activity of kombucha fermented milk products with stinging nettle and winter savory. Chemical Industry and Chemical Engineering Quarterly, 19(1): 129-139. http://dx.doi.org/10.2298/CICEQ120205048V

Watawana MI, Jayawardena N, Gunawardhana CB and Waisundara VY. 2015. Health, wellness, and safety aspects of the consumption of kombucha. Journal of Chemistry, 11. https://doi.org/10.1155/2015/591869

Wong-Paz JE, Muñiz-Márquez DB, Aguilar-Zárate P, Rodríguez-Herrera R and Aguilar CN. 2014. Microplate quantification of total phenolic content from plant extracts obtained by conventional and ultrasound methods. Phytochemical Analysis: PCA, 25(5): 439-444. https://doi.org/10.1002/pca.2512

Yang X, Wang K, Zhang J, Tang L and Mao Z. 2016. Effect of acetic acid in recycling water on ethanol production for cassava in an integrated ethanol-methane fermentation process. Water Science and Technology: A Journal of the International Association on Water Pollution Research, 74(10): 2392-2398. https://doi.org/10.2166/wst.2016.228