

Influence of carbon sources on the physicochemical and antioxidant properties of pitaya (*Stenocereus* sp.) wines

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Abstract. Pitaya (*Stenocereus* sp.) is recognized for its high antioxidant potential and abundance of bioactive compounds. The aim of this study was to evaluate the effects of supplementing pitaya must with different carbon sources on fermentation performance, physicochemical characteristics, and antioxidant capacity to identify the most suitable formulation for pitaya wine production. Pitaya juices were adjusted to 22 °Brix and fermented with *Saccharomyces cerevisiae* at 27 °C for 9 days. Fermentation was assessed by soluble solids (°Brix), total reducing sugars (TRS), ethanol production, fermentation yield, and fermentation efficiency. The final beverages were characterized by color, total phenolic content, betalains, and antioxidant capacity using DPPH, ABTS, and FRAP assays. Significant differences ($p < 0.05$) were observed among carbon sources. All treatments showed a marked decline in °Brix during the first 3-5 days. Pitaya supplemented with agave syrup (AASP) exhibited the highest fermentative performance, reaching the highest ethanol concentration (84.16 g L⁻¹), fermentation yield (0.46 g ethanol/g sugar consumed), and fermentation efficiency (89.71%). The FRAP and ABTS assays showed that AASP after fermentation exhibited the highest antioxidant capacity, with values of 640.90 ± 2.57 and $11,568.33 \pm 147.99$ µmol TE mL⁻¹, respectively, whereas the greatest DPPH scavenging activity was observed in agave syrup after fermentation (AAS) (632.11 ± 12.62 µmol TE mL⁻¹). The highest total phenolic content was observed in agave syrup with pitaya before fermentation (BASP), with a value of 404.94 ± 1.85 mg GAE L⁻¹. These findings demonstrate that agave syrup is the most suitable carbon source for producing fermented pitaya beverages with high fermentative efficiency while preserving their antioxidant potential.

Keywords: agave syrup, blossom honey, brown cane sugar, fermentation

Introduction

Pitaya (*Stenocereus* sp.), a fruit native to the Cactaceae family, is widely cultivated in Mexico and has long been appreciated for its nutritional value and potential health-promoting properties (Ramírez-Rodríguez *et al.*, 2020). Its composition is characterized by the presence of bioactive compounds, including betalains, phenolic compounds, and vitamins, which contribute to its antioxidant capacity (García-Cruz *et al.*, 2017).

In addition to its nutritional properties, *Stenocereus* pitaya has been recognized as an underutilized fruit with considerable nutraceutical and agroindustrial potential, particularly because its betalain-rich tissues represent a promising source of natural pigments for food applications (Quiroz-González et al., 2018). In parallel with the growing consumer interest in fruit-based alcoholic beverages, research has increasingly focused on the use of non-conventional fruits as substrates for wine production (Tsegay et al., 2018). Among these alternatives, cactus fruits have attracted attention because of their natural sugar content and their abundance of antioxidant compounds. Recent studies on dragon fruit wines have concentrated on optimizing the conditions of fermentation, the physicochemical characterization, sensory qualities and the antioxidant potential (Gong et al., 2017; Hu et al., 2023; Anh et al., 2025).

For example, Anh et al. (2025) reported that red dragon fruit wine fermented with *Saccharomyces cerevisiae* exhibited considerable concentrations of phenolic compounds, betalains, and antioxidant activity. Likewise, Bunga et al. (2025) demonstrated the feasibility of producing a functional wine from a blend of red dragon fruit (*Hylocereus polyrhizus*) juice and palmyra neera, obtaining a fermented beverage with acceptable physicochemical characteristics and antioxidant activity. In addition, mixtures of blossom honey and sugar have been used in the fermentation of prickly pear (*Opuntia ficus-indica*) to produce bio-functional alcoholic beverages (Karabagias et al., 2020).

Previous studies have focused on the optimization of cactus fruit wine production. For example, Tsegay et al. (2018) optimized the fermentation of cactus pear (*Opuntia* spp.) wines by investigating the effects of inoculum concentration, temperature, and pH on the fermentation process. Furthermore, the influence of carbon sources on the quality of fermented beverages has attracted increasing attention in recent years. Beyond serving as substrates for microbial growth and ethanol production, sweeteners may significantly affect the chemical composition and functional properties of the final product. For instance, Gülhan (2023) reported that the type of carbon source used in water kefir-based lemonade formulations influenced antioxidant activity, microbial development, vitamin C retention, mineral composition, and sensory acceptance. These findings suggest that carbon sources should not be regarded solely as providers of fermentable sugars, but also as ingredients capable of modifying the nutritional and bioactive characteristics of fermented beverages.

The potential impact of different sweeteners is closely related to their chemical composition. Refined brown cane sugar is composed predominantly of sucrose (98.64 ± 0.51 g/100 g), with only minor amounts of glucose and fructose (Lee et al., 2018). In contrast, honey contains higher proportions of reducing sugars, mainly fructose (35.6-41.8 g/100 g) and glucose (25.4-28.1 g/100 g), together with trace amounts of minerals, organic acids, enzymes, and phenolic compounds that may contribute to antioxidant activity (Cianciosi et al., 2018).

Agave syrup is also notably different from cane sugar as it is predominantly composed of fructose ($84.29 \pm 5.58\%$) and glucose ($8.33 \pm 2.87\%$) with sucrose only found in traces (Willems and Low, 2012). These compositional differences show that each sweetener may offer different fermentation substrates and bioactive elements that may influence yeast metabolism, phenolic retention and antioxidant capacity in the finished wine. Honey also has a natural sweetening property and contains substantial bioactive chemicals such as phenolic acids and flavonoids, which contribute to its antioxidant profile (Mendoza-Bacilio et al., 2022). However, few studies have investigated the use of agave syrup as a carbon source in fruit wine production.

Agave-derived products have gained increasing attention due to their nutritional value and the presence of bioactive compounds with potential health-promoting properties. “Aguamiel”, the sap used for agave syrup production, has been recognized as a functional beverage because of its content of bioactive constituents and its antioxidant capacity (Hernández-Ramos *et al.*, 2025). These characteristics suggest that agave syrup may contribute not only fermentable sugars for yeast metabolism but also antioxidant compounds that could influence the functional quality of fermented beverages. Despite these promising attributes, limited information is available regarding the effect of agave syrup on the physicochemical characteristics and antioxidant-related properties of pitaya wines. Therefore, further research is needed to elucidate its potential role in enhancing the quality and bioactive profile of these fermented products.

Few comparative studies have been conducted with honey, agave syrup and cane sugar as fermentation substrates for betalain-rich fruit wines. This knowledge gap limits our understanding of how the carbon source composition may influence the functional quality of pitaya wine and its potential as a value-added fermented beverage. Therefore, the assessment of alternative carbon sources could help to produce fermented beverages with enhanced functional properties and added value. The aim of this study was to evaluate the effects of supplementing pitaya (*Stenocereus* sp.) with different carbon sources (blossom honey, agave syrup, and refined brown cane sugar) on fermentation performance, physicochemical characteristics, and antioxidant capacity to identify the most suitable formulation for pitaya wine production.

Materials and Methods

Preparation of pitaya (*Stenocereus* sp.)

The red pitaya (*Stenocereus* sp.) from Rioverde, San Luis Potosí, Mexico, was harvested, cooled, and processed within 12 h to minimize the effect of postharvest handling (10 kg). The fruits were washed with tap water and treated with a detergent Salvo® (Ciudad de México, México). Then the spines and peels were manually removed. The juice was obtained by manual maceration of the pulp of the pitaya and filtration through cheesecloth.

Fermentation process

After juice preparation, blossom honey (Grupo Colibrí, San Andrés de la Cal, Tepoztlán, Morelos, Mexico), agave syrup (Jaguey, Nopala, Hidalgo, Mexico), and cane sugar (azúcar estandar, La Posada®, Monterrey; Mexico) were added separately to adjust the soluble solids content of pitaya juice to 22 °Brix, generating the ABHP (pitaya juice supplemented with blossom honey), AASP (pitaya juice supplemented with agave syrup), AACP (pitaya juice supplemented with cane sugar) formulations. In addition, control fermentations consisting exclusively of blossom honey (BBH), agave syrup (BAS), or cane sugar (BAC) adjusted to 22 °Brix with distilled water were prepared under the same experimental conditions. The initial soluble solids content was selected because it is commonly used in fruit wine production to provide an adequate concentration of fermentable sugars, resulting in an ethanol content comparable to that of table wines (approximately 10-13% v/v) (Jackson, 2020).

Fermentation was carried out in 500 mL flasks with a working volume of 450 mL, and all treatments were prepared in triplicate. To ensure comparable fermentation conditions, yeast nutrient (NutriBrew, Hazchela, Mexico) was added to all treatments at a concentration of 150 mg L⁻¹, together with sodium metabisulfite (70 mg L⁻¹; Mi Granero, Mexico), prior to yeast inoculation. The yeast strain

Saccharomyces cerevisiae Premier Classique (Red Star, Gen, Belgium) was inoculated at 373 mg per flask. Fermentations were conducted in the dark. Fermentations were performed in a temperature controlled incubator (Binder, Tuttlingen, Germany). The fermentation temperature was maintained at 27 °C, a condition commonly used for *Saccharomyces cerevisiae* in fruit wine fermentation to promote efficient sugar conversion while minimizing thermal stress in yeast cells (Tsegay *et al.*, 2018; Anh *et al.*, 2025).

Fermentation progress was monitored daily by measuring soluble solids (°Brix). Based on previous reports on cactus pear and dragon fruit wines, fermentation periods of approximately 6 days have been described (Tsegay *et al.*, 2018; Anh *et al.*, 2025). In the present study, fermentations were maintained for 9 days to standardize the process across all treatments under the evaluated experimental conditions.

Total reducing sugar (TRS) determination

The initial and final concentrations of total reducing sugars (TRS) were determined in triplicate according to the Mexican Standard NMX-V-006-NORMEX-2013 using the Lane-Eynon volumetric titration method. The results were expressed as g L⁻¹.

Ethanol content determination

Ethanol content was determined according to the Mexican Standard NMX-V-013-NORMEX-2013. Briefly, a 50 mL aliquot of each fermented sample was transferred to a 250 mL distillation flask. The original volumetric flask was rinsed with distilled water, and the rinsing was added to the distillation flask. The sample was distilled, and the distillate was collected in a 50 mL volumetric flask containing approximately 4 mL of distilled water. After cooling, the volume was adjusted to the mark with distilled water. Ethanol concentration was determined using a portable alcohol refractometer (RHW-80ATC, 0-80% v/v), expressed as % (v/v), and subsequently converted to grams per liter (g L⁻¹) using the density of ethanol at 20 °C (0.789 g mL⁻¹).

Total phenolic content

The total phenolic content was assessed using the colorimetric Folin-Ciocalteu technique, following the protocol outlined by Everette *et al.* (2010), with minor adjustments. A standard solution of gallic acid (0.1 mg mL⁻¹) was prepared, and the Folin reagent was diluted by combining 10 mL of Folin-Ciocalteu reagent with 10 mL of distilled water. A 20% sodium carbonate (Na₂CO₃) solution was made by dissolving 20 g of Na₂CO₃ in 100 mL of distilled water. For the samples, 500 µL of the extract was utilized immediately. In both the standards and samples, 250 µL of diluted Folin reagent and 1,250 µL of Na₂CO₃ solution were combined to achieve a final volume of 2 mL. The mixtures were incubated in darkness for 2 h at ambient temperature. Absorbance was subsequently quantified at 760 nm.

Ferric reducing antioxidant power (FRAP)

The antioxidant capacity of each clarified juice was determined by a modified FRAP method. The FRAP reagent was prepared by mixing three solutions: (1) a 300 mM acetate buffer (pH 3.6) prepared from sodium acetate trihydrate, glacial acetic acid and distilled water; (2) a TPTZ (2,4,6-tripyridyl-s-triazine) solution prepared in concentrated HCl and distilled water; and (3) a FeCl₃·6H₂O solution dissolved in Milli-Q water. The components were mixed at a ratio of 10:1:1 and incubated at 30 °C for 30 min in the dark. Then, 100 µL of each clarified juice sample was mixed with 3 mL of the FRAP

reagent. Absorbance was read at 593 nm. All measurements were carried out in triplicate. The assay was performed using the technique of Benzie and Strain (1996).

ABTS•+ radical scavenging assay

The antioxidant capacity of wines was assessed using the ABTS•+ assay according to a modified protocol described by Re *et al.* (1999). To create the ABTS•+ radical solution, 77.6 mg of ABTS was solubilized in 20 mL of double-distilled water. Thereafter, 13.2 mg of potassium persulfate was incorporated into the ABTS solution within an amber bottle. The mixture was homogenized, covered with aluminum foil, and allowed to react in the dark at room temperature for 16 hours to produce the radical cation. The solution was further diluted with 100% ethanol to achieve an absorbance of 0.70 ± 0.02 at 732 nm. A 4 mM Trolox stock solution was produced for the calibration curve. The absorbance of each standard was recorded 6 minutes post-mixing with the ABTS•+ solution. To assess the antioxidant capacity of the samples, 10 μ L of each sample was combined with 1 mL of ABTS•+ solution. Following a reaction period of 6 minutes, absorbance was measured at 732 nm.

DPPH• radical scavenging activity

The antioxidant capacity of the samples was assessed using the DPPH• test, following the methodology outlined by Brand-Williams *et al.* (1995), with some modifications. The DPPH• solution was formulated in 80% methanol and then diluted at a 1:10 ratio to attain an absorbance of 0.7 at 515 nm. Trolox served as the standard for the calibration curve. Results were presented as Trolox equivalents (TE).

Quantification of betacyanins and betaxanthins

Betacyanins and betaxanthins were quantified spectrophotometrically using a SmartSpec® Plus spectrophotometer, following the method described by Nilsson (1970) and Sandate-Flores *et al.* (2016), with modifications. Distilled water was used for the necessary dilutions. The concentrations of betacyanins (β C, mg/L) and betaxanthins (β X, mg/L) were determined using Equation 1.

$$BC = \frac{Ax D_f x MW x 1000}{E_{1\%} L} \quad [1]$$

$E_{1\%}$ refers to the molar extinction coefficient: 60,000 L mol⁻¹ cm⁻¹ for betacyanins and 48,000 L mol⁻¹ cm⁻¹ for betaxanthins. A is the absorbance measured at 540 nm for betacyanins and 480 nm for betaxanthins. D_f is the dilution factor applied to the sample. MW represents the molecular weight of the representative compound. L is the path length of the cuvette, fixed at 1 cm.

Color measurement

Color was determined using the CIE system parameters: L* (0 = black; 100 = white), a* (– = green; + = red), and b* (– = blue; + = yellow), as well as Chroma (C*, color intensity) and Hue angle (h°, hue or shade), employing a colorimeter (CR-410, Konica Minolta, Japan).

Experimental treatment combinations evaluated in this study

The experiment was conducted to evaluate the effects of different carbon sources on the fermentation performance, physicochemical characteristics, and antioxidant capacity of pitaya (*Stenocereus* sp.) wine. The experimental treatments consisted of fermentations prepared with or without pitaya juice

using blossom honey, agave syrup, or refined brown cane sugar as carbon sources. Samples were analyzed before and after fermentation, resulting in the treatment combinations summarized in Table 1. All fermentations were performed in triplicate, and each treatment was considered an independent experimental unit for statistical analysis.

Table 1. Experimental treatment combinations used to evaluate the effects of carbon source supplementation on pitaya wine production.

Treatment code	Carbon source	Pitaya juice	Fermentation stage
BBH	Blossom honey	Absent	Before fermentation
ABH	Blossom honey	Absent	After fermentation
BAS	Agave syrup	Absent	Before fermentation
AAS	Agave syrup	Absent	After fermentation
BAC	Brown cane sugar	Absent	Before fermentation
AAC	Brown cane sugar	Absent	After fermentation
BBHP	Blossom honey	Present	Before fermentation
ABHP	Blossom honey	Present	After fermentation
BASP	Agave syrup	Present	Before fermentation
AASP	Agave syrup	Present	After fermentation
BACP	Brown cane sugar	Present	Before fermentation
AACP	Brown cane sugar	Present	After fermentation

Statistical analysis

Each response variable was analyzed independently using one-way analysis of variance (ANOVA). Fermentation performance variables were analyzed considering the six fermented treatments (AASP, ABHP, AACP, AAS, ABH, and AAC), whereas physicochemical characteristics, total phenolic content, betalains, and antioxidant capacity were analyzed considering all treatment combinations before and after fermentation. Prior to the analysis, the assumptions of the general linear model were verified. The independence of observations was ensured through the experimental design, in which each fermentation replicate was conducted independently. The normality of residuals was assessed using the Shapiro-Wilk test, whereas homogeneity of variances was evaluated using Levene's test. Variables that did not satisfy the assumptions of normality and homogeneity of variances ($p < 0.05$) were log10-transformed and reassessed before ANOVA. When significant differences among treatments were detected ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) test was applied for pairwise comparisons of treatment means. All statistical analyses were conducted at a significance level of $\alpha = 0.05$. Statistical analyses were performed using the transformed data when required, whereas results are presented on the original scale to facilitate biological interpretation. Additionally, Principal Component Analysis (PCA) was performed to explore multivariate relationships among the evaluated variables and treatments. All statistical analyses were conducted using InfoStat software (version 2020, Universidad Nacional de Córdoba, Córdoba, Argentina).

Results and Discussion

The evolution of soluble solids (°Brix) during fermentation is shown in Figure 1, whereas total reducing sugar (TRS), ethanol production, fermentation yield, and fermentation efficiency are summarized in Table 2. The marked decline in soluble solids (°Brix) during the first three to five days indicates the period of greatest fermentative activity. Similar fermentation kinetics have been reported for fruit wines,

in which the rapid metabolism of fermentable sugars occurs during the exponential growth phase of *Saccharomyces cerevisiae* (Walker and Stewart, 2016; Jackson, 2020).

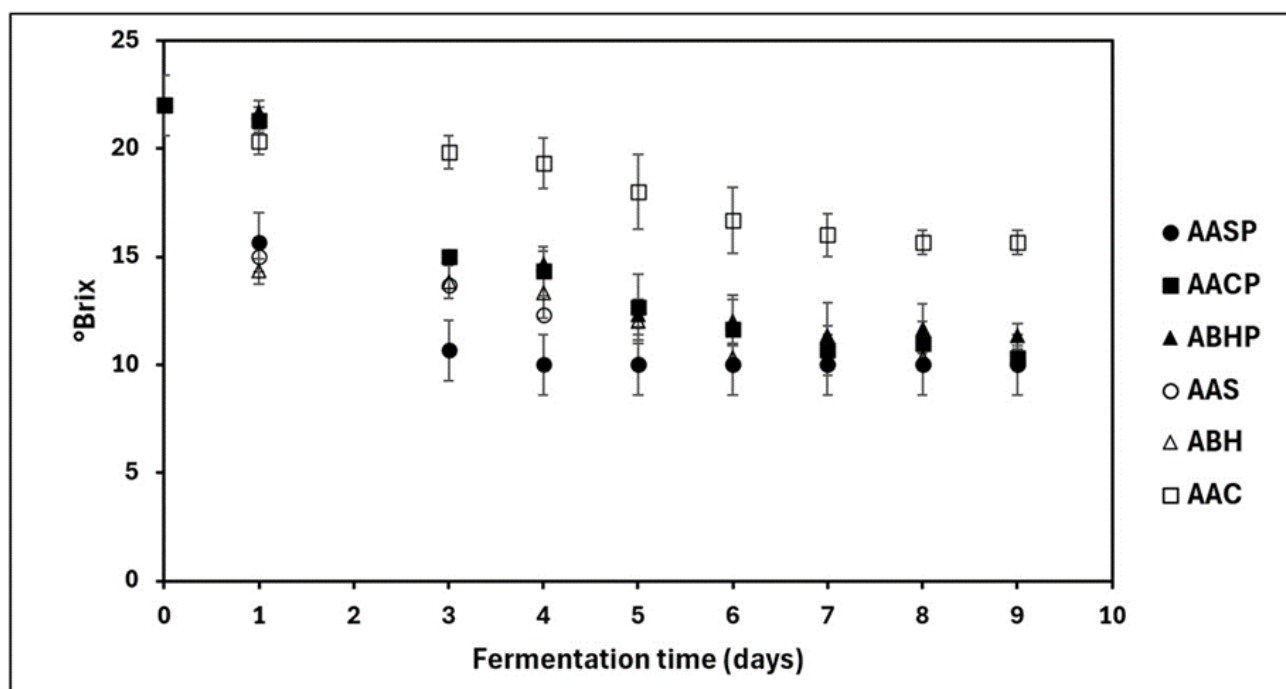


Figure 1. Changes in soluble solids (°Brix) during 9 days of alcoholic fermentation of pitaya juice supplemented with agave syrup, blossom honey, or brown cane sugar, and the corresponding control formulations. AASP, pitaya supplemented with agave syrup; ABHP, pitaya supplemented with blossom honey; AACP, pitaya supplemented with brown cane sugar; AAS, agave syrup; ABH, blossom honey; AAC, brown cane sugar. Values represent the mean $\pm$ standard deviation ($n = 3$).

Table 2. Ethanol concentration, sugar consumption, fermentation yield, and efficiency of the evaluated treatments

Treatments	Ethanol concentration (g L ⁻¹)	Initial TRS (g L ⁻¹)	Final TRS (g L ⁻¹)	Yield (g g ⁻¹)	Efficiency (%)
AASP	84.16 $\pm$ 4.55 ^{c*}	221.29 $\pm$ 0.99 ^a	37.37 $\pm$ 1.92 ^a	0.46 $\pm$ 0.02 ^b	89.71 $\pm$ 4.54 ^b
ABHP	68.38 $\pm$ 4.55 ^{bc}	223.67 $\pm$ 1.09 ^a	50.92 $\pm$ 1.19 ^c	0.39 $\pm$ 0.03 ^{ab}	77.64 $\pm$ 5.68 ^{ab}
AACP	65.75 $\pm$ 12.05 ^b	224.96 $\pm$ 0.34 ^a	48.93 $\pm$ 1.08 ^c	0.37 $\pm$ 0.07 ^{ab}	73.29 $\pm$ 13.85 ^{ab}
AAS	73.64 $\pm$ 4.55 ^{bc}	223.01 $\pm$ 1.51 ^a	40.67 $\pm$ 2.24 ^{ab}	0.40 $\pm$ 0.03 ^{ab}	79.21 $\pm$ 5.30 ^{ab}
ABH	65.75 $\pm$ 4.55 ^b	220.95 $\pm$ 1.92 ^a	47.10 $\pm$ 3.25 ^{bc}	0.38 $\pm$ 0.03 ^{ab}	74.19 $\pm$ 5.73 ^{ab}
AAC	34.19 $\pm$ 4.55 ^a	226.63 $\pm$ 0.93 ^a	130.93 $\pm$ 5.48 ^d	0.32 $\pm$ 0.04 ^a	63.63 $\pm$ 8.63 ^a

*Values are expressed as mean $\pm$ standard deviation ($n = 3$). Different superscript letters within the same column indicate significant differences among treatments according to Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$). TRS: total reducing sugars. Yield was calculated as grams of ethanol produced per gram of sugar consumed. Fermentation efficiency was calculated relative to the theoretical ethanol yield of 0.51 g ethanol/g sugar. AASP: pitaya supplemented with agave syrup, ABHP: pitaya supplemented with blossom honey, AACP: pitaya supplemented with brown cane sugar, AAS: agave syrup, ABH: blossom honey, AAC: brown cane sugar.

Although all formulations were adjusted to similar initial TRS concentrations (220.95-226.63 g L⁻¹), significant differences ($p < 0.05$) were observed in sugar utilization and fermentative performance. AASP (pitaya juice supplemented with agave syrup) exhibited the greatest sugar consumption, with TRS decreasing from 221.29 to 37.37 g L⁻¹ (Table 2) and reached one of the lowest final °Brix values (≈10 °Brix). In contrast, AAC (brown cane sugar) retained significantly higher residual soluble solids (≈15.5 °Brix) and TRS (130.93 g L⁻¹), indicating incomplete substrate utilization. These differences were reflected in ethanol production, as AASP produced the highest ethanol concentration (84.16 g L⁻¹), fermentation yield (0.46 g ethanol per g of sugar consumed), and fermentation efficiency (89.71%), whereas AAC showed the lowest values for all three parameters (Table 2).

Both agave syrup and blossom honey supported efficient alcoholic fermentation, with no significant differences in ethanol production, fermentation yield, or fermentation efficiency between their corresponding formulations. Nevertheless, agave syrup consistently produced numerically higher values than blossom honey, both in the presence and absence of pitaya juice. Agave syrup and blossom honey contain high proportions of fructose and glucose, monosaccharides that are readily transported and metabolized by *Saccharomyces cerevisiae*. In contrast, sucrose (brown cane sugar) requires extracellular hydrolysis by invertase before entering glycolysis, increasing the metabolic demand on yeast cells (Bogdanov et al., 2008; Mellado-Mojica and López, 2015; Walker and Stewart, 2016). This difference may have contributed to the greater sugar conversion and ethanol production observed in the formulations containing agave syrup and blossom honey compared with the brown cane sugar.

AASP and AAS exhibited statistically similar ethanol production, fermentation yield, and fermentation efficiency (Table 2), indicating that agave syrup was the primary factor governing fermentative performance under the evaluated conditions. Both treatments showed greater sugar utilization and ethanol production than the AAC treatment (Table 2), highlighting the favorable effect of fructose-rich substrates on *Saccharomyces cerevisiae* metabolism (Mellado-Mojica and López, 2015; Walker and Stewart, 2016). Although pitaya supplementation did not significantly enhance fermentation efficiency relative to agave syrup alone, it did not impair fermentative performance and contributed to the functional properties of the final beverage, as evidenced by its higher antioxidant capacity.

It should also be noted that refractometric measurements represent total soluble solids rather than fermentable sugars exclusively; therefore, residual °Brix includes organic acids, minerals, pectins, phenolic compounds, and other non-fermentable constituents (Jackson, 2020). Nevertheless, the agreement between the high residual °Brix, elevated TRS concentration, low ethanol production, and reduced fermentation efficiency in AAC confirms that sucrose was less efficiently converted into ethanol under the evaluated conditions. Taken together, these findings demonstrate that agave syrup is a suitable carbon source for pitaya wine production, promoting efficient sugar conversion and ethanol production. The high fermentation efficiency achieved by the AASP formulation (89.71%), which approached the theoretical maximum ethanol yield (0.51 g ethanol per g of sugar), underscores its suitability for producing pitaya wine under the conditions evaluated.

The ethanol concentrations obtained in the present study are consistent with those reported for fruit wines, which typically contain 8-14% (v/v) alcohol depending on the fruit matrix, initial sugar concentration, and fermentation conditions (Jackson, 2020). Based on the measured ethanol

concentrations, AASP reached an estimated alcohol content of approximately 10.7% (v/v), which falls within the range of conventional table wines, whereas AAS, ABHP, AACP, and ABH produced beverages containing approximately 8.3-9.3% (v/v) alcohol, comparable to many commercial table fruit wines.

The evaluation of antioxidant capacity and total polyphenol content (TPC) across the different treatments revealed clear patterns influenced by both the type of carbon source employed and the addition of pitaya (Table 3). The analysis of variance revealed statistically significant differences ($p < 0.05$) in the antioxidant capacity of the wine samples, attributable to both the type of carbon source used ($p = 0.001$) and the addition of pitaya ($p = 0.003$). Although the absolute values obtained using the DPPH, ABTS, and FRAP assays differed from each other, all methods showed the same overall trend, consistently identifying the samples with the highest and lowest antioxidant activity. Among them, the ABTS assay demonstrated a greater ability to discriminate between treatments.

Agave syrup-based treatments exhibited the highest total phenolic contents and antioxidant capacities among the evaluated wines (Tables 3 and 4). Agave syrup with pitaya before fermentation (BASP) showed the highest total phenolic content (404.94 ± 1.85 mg GAE L⁻¹), followed by AASP (234.37 ± 1.85 mg GAE L⁻¹) and AACP (227.48 ± 1.85 mg GAE L⁻¹), with no significant difference observed between the latter two treatments. In contrast, the highest antioxidant capacity (FRAP method) was observed in AASP, followed by AAS, BAS, and BASP. These results suggest that, although fermentation reduced the total phenolic content, the antioxidant capacity remained high, likely due to the contribution of betalains and other antioxidant compounds preserved or transformed during fermentation. Similar findings have been reported in other fermented products rich in bioactive compounds. Therefore, the antioxidant activity observed in the present study was compared with that of other fermented betalain-rich matrices to better contextualize the contribution of phenolic compounds and betalains to the antioxidant properties of the wines.

Table 3. Antioxidant capacity and total phenolic content of wines produced with different carbon sources before and after fermentation.

Treatment	FRAP ($\mu\text{mol TE mL}^{-1}$)	ABTS ($\mu\text{mol TE mL}^{-1}$)	DPPH ($\mu\text{mol TE mL}^{-1}$)	TPC (mg GAE L ⁻¹)
AAS	$625.46 \pm 0.74^{\text{a*}}$	$5276.67 \pm 69.24^{\text{a}}$	$632.11 \pm 12.62^{\text{a}}$	$54.50 \pm 1.14^{\text{b}}$
BAS	$624.16 \pm 0.74^{\text{a}}$	$4935.00 \pm 69.24^{\text{b}}$	$605.44 \pm 12.62^{\text{a}}$	$64.81 \pm 1.14^{\text{a}}$
BAC	$493.91 \pm 0.74^{\text{b}}$	$479.33 \pm 69.24^{\text{c}}$	$8.1 \pm 12.62^{\text{c}}$	$8.79 \pm 1.14^{\text{e}}$
ABH	$393.73 \pm 0.74^{\text{c}}$	$226.67 \pm 69.24^{\text{de}}$	$71.88 \pm 12.62^{\text{b}}$	$52.38 \pm 1.14^{\text{bc}}$
AAC	$389.55 \pm 0.74^{\text{d}}$	$118.50 \pm 69.24^{\text{e}}$	$73.66 \pm 12.62^{\text{b}}$	$48.67 \pm 1.14^{\text{c}}$
BBH	$308.99 \pm 0.74^{\text{e}}$	$751.67 \pm 69.24^{\text{c}}$	$32.77 \pm 12.62^{\text{bc}}$	$22.83 \pm 1.14^{\text{d}}$
<i>p-value</i>	0.0001	0.0001	0.0001	0.0001

*Values are expressed as mean $\pm$ standard error ($n = 3$). Different superscript letters within the same column indicate significant differences according to Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$). FRAP: Ferric reducing antioxidant power, ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl, TE: Trolox equivalents, TPC: Total phenolic content expressed as mg GAE L⁻¹, GAE: gallic acid equivalents. AAS: agave syrup after fermentation, ABH: blossom honey after fermentation, AAC: brown cane sugar after fermentation, BAS: agave syrup before fermentation, BBH: blossom honey before fermentation, BAC: brown cane sugar before fermentation.

Table 4. Antioxidant capacity and total phenolic content of pitaya wines produced with different carbon sources before and after fermentation.

Treatment	FRAP ($\mu\text{mol TE mL}^{-1}$)	ABTS ($\mu\text{mol TE mL}^{-1}$)	DPPH ($\mu\text{mol TE mL}^{-1}$)	TPC (mg GAE L^{-1})
AASP	640.90 $\pm$ 2.57 ^{a*}	11568.33 $\pm$ 147.99 ^a	477.67 $\pm$ 10.85 ^a	234.37 $\pm$ 1.85 ^b
BASP	613.43 $\pm$ 2.57 ^b	3598.75 $\pm$ 147.99 ^d	416.56 $\pm$ 10.85 ^b	404.94 $\pm$ 1.85 ^a
AACP	486.06 $\pm$ 2.57 ^c	4501.67 $\pm$ 147.99 ^c	156.1 $\pm$ 10.85 ^c	227.48 $\pm$ 1.85 ^b
BACP	469.84 $\pm$ 2.57 ^d	2259.17 $\pm$ 147.99 ^e	99.66 $\pm$ 10.85 ^{de}	64.99 $\pm$ 1.85 ^d
ABHP	437.94 $\pm$ 2.57 ^e	6401.67 $\pm$ 147.99 ^b	148.32 $\pm$ 10.85 ^{cd}	149.61 $\pm$ 1.85 ^c
BBHP	401.06 $\pm$ 2.57 ^f	2326.67 $\pm$ 147.99 ^e	51.66 $\pm$ 10.85 ^e	46.58 $\pm$ 1.85 ^e
<i>p-value</i>	0.0001	0.0001	0.0001	0.0001

*Values are expressed as mean $\pm$ standard error ($n = 3$). Different superscript letters within the same column indicate significant differences according to Tukey's Honestly Significant Difference (HSD) test ($p < 0.05$). FRAP: Ferric reducing antioxidant power, ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl, TE: Trolox equivalents, TPC: Total phenolic content expressed as mg GAE L^{-1} , GAE: gallic acid equivalents. AASP: pitaya supplemented with agave syrup after fermentation, ABHP: pitaya supplemented with blossom honey after fermentation, AACP: pitaya supplemented with brown cane sugar after fermentation, BASP: pitaya supplemented with agave syrup before fermentation, BBHP: pitaya supplemented with blossom honey before fermentation, BACP: pitaya supplemented with brown cane sugar before fermentation.

For example, beetroot wine retained substantial levels of betalains and antioxidant activity after fermentation (Singh *et al.*, 2021), while fermented beetroot juices showed a strong positive correlation between FRAP values and total phenolic content (Jakubczyk *et al.*, 2024). Furthermore, the total phenolic content of the blossom honey wine without pitaya was lower than that reported for multifloral mead (218.30 ± 3.56 mg GAE L^{-1}) by Fortes *et al.* (2023) and for mead produced by Akalın *et al.* (2017) (167.89 mg GAE L^{-1}), suggesting that the addition of pitaya and the use of agave syrup may enhance the phenolic profile of fermented beverages beyond that typically observed in conventional honey-based meads. The total phenolic content reported by Karabagias *et al.* (2020) for a bio-functional alcoholic beverage prepared from prickly pear juice and pulp supplemented with sugar and blossom honey ($9,800 \pm 5,500$ mg GAE L^{-1}) was substantially higher than that observed in the present study for wines produced with blossom honey and pitaya.

This difference may be related to variations in fruit composition, formulation, processing conditions, and the proportion of fruit solids incorporated into the fermentation matrix. Similarly, although information regarding wines produced with agave syrup is scarce, Hernández-Ramos *et al.* (2025) reported a total phenolic content of 478.28 ± 20.94 mg GAE L^{-1} in aguamiel from *Agave salmiana*, the raw material used for agave syrup production. The lower phenolic values observed in agave syrup-based wines were expected because the manufacture of agave syrup involves thermal concentration processes that may promote the degradation of thermolabile compounds, including polyphenols. The wines produced with agave syrup and brown cane sugar also exhibited a decrease in total phenolic content after fermentation, a trend that has likewise been reported in wines produced from *Ziziphus jujuba* Mill. (Yuan *et al.*, 2022) and *Hylocereus costaricensis* (Hu *et al.*, 2023).

Although the present study involved a betalain-rich matrix rather than an anthocyanin-rich one, similar reductions in pigment-associated phenolic compounds have been reported during wine fermentations with *Saccharomyces cerevisiae*. Morata *et al.* (2003) demonstrated that anthocyanins can be adsorbed onto yeast cell walls during red wine fermentation, thereby reducing their concentration in the final

product. Likewise, Echeverrigaray *et al.* (2020) reported that anthocyanin adsorption by *Saccharomyces cerevisiae* is associated with changes in cell wall integrity during fermentation. Since both anthocyanins and betalains are water-soluble pigments that contribute to antioxidant activity and color, adsorption and interaction phenomena between yeast cells and pigment-related compounds may partially contribute to the reduction in total phenolic content observed after fermentation. However, further studies are required to confirm whether similar mechanisms occur in betalain-rich pitaya wines. In contrast, wines produced with blossom honey showed an increase in total phenolic content after fermentation.

Similar behavior has been reported in meads, where fermentation promoted increases in phenolic compounds, possibly due to the release of bound phenolics and changes in their extractability (Adamenko *et al.*, 2021; Fu *et al.*, 2022). These findings suggest that the evolution of phenolic compounds during fermentation is strongly influenced by the chemical composition of the carbon source rather than by fermentation alone. Despite the reduction observed in some treatments, phenolic compounds, betalains, and other bioactive constituents collectively contributed to the antioxidant potential of final products.

The antioxidant activity observed in the present study was notable when compared with values reported for commercial white wines. Specifically, the blossom honey and agave syrup-based wines exhibited higher antioxidant activity as determined by the ABTS assays than that reported for a white wine ($460 \pm 320 \mu\text{M TE}$) by Villaño *et al.* (2004). Likewise, the agave syrup-based wine without pitaya showed greater antioxidant activity according to the DPPH assay than Chenin Blanc white wines ($544 \pm 200 \mu\text{M TE}$) reported by De Beer *et al.* (2003). These findings indicate that the antioxidant potential of the experimental wines is comparable to or greater than that of certain commercial white wines. However, the wine exhibiting the highest antioxidant activity varied depending on the assay employed, suggesting that the antioxidant response depends on the specific chemical characteristics of the compounds present and the analytical principles underlying each assay. A robust positive correlation was noted between total polyphenol content and antioxidant activity throughout the samples, regardless of the assay used (DPPH, ABTS, or FRAP) (Pyrzynska and Sentkowska, 2023).

This association was further supported by the principal component analysis (PCA) (Figure 2), which explained 86.2% of the total variability through the first two components: PC1 (69.6%) and PC2 (16.6%). Variables such as TPC, DPPH, FRAP, βC , and βX showed vectors oriented in similar directions, indicating a joint contribution to PC1 and reinforcing their strong positive association. In contrast, ABTS loaded predominantly on PC2, suggesting that this method captures a distinct dimension of the antioxidant system. The projection of samples in the bidimensional space revealed clear groupings, with AASP and BASP positioned in the quadrant associated with high antioxidant and pigment levels. Meanwhile, AACP and BACP exhibited balanced profiles with intermediate values, and BBHP and ABHP were placed in the opposite quadrant, reflecting their lower contribution in terms of both antioxidant capacity and pigmentation.

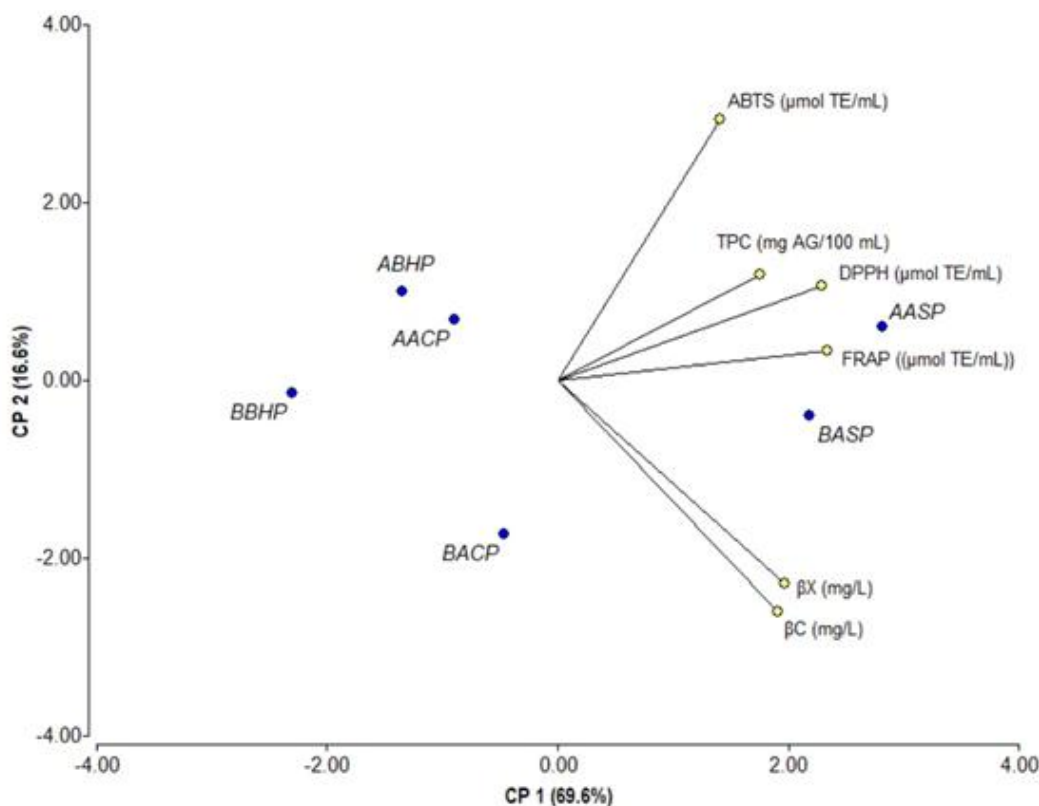


Figure 2. Principal component analysis (PCA) showing the relationships among total phenolic content expressed as mg GAE L⁻¹, antioxidant activity (DPPH, ABTS, FRAP), and betalain pigments (βC, βX), and pitaya wines produced with different carbon sources. FRAP: Ferric reducing antioxidant power, ABTS: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), DPPH: 2,2-diphenyl-1-picrylhydrazyl, βC: betacyanins, βX: betaxanthins, TE: Trolox equivalents, TPC: Total phenolic content, GAE: Gallic acid equivalents.

Betacyanins showed a strong positive correlation with the color parameter a^* ($r = 0.75$) and a moderate correlation with c^* ($r = 0.63$), suggesting that these pigments contribute significantly to the development of red-violet hues and the chromatic intensity of the wine (Table 5). Likewise, betaxanthins exhibited strong positive correlation with a^* ($r = 0.79$), b^* ($r = 0.81$), and c^* ($r = 0.85$), suggesting that these pigments contribute not only to yellow coloration but also to the overall color intensity of the fermented beverages. Both betalain groups were negatively correlated with lightness (L^*) ($r = -0.68$ for betacyanins and $r = -0.87$ for betaxanthins), indicating that increasing pigment concentrations were associated with darker wines. These results are consistent with the role of betalains as the principal pigments responsible for the visual characteristics of pitaya-derived products and support their contribution to the color attributes observed after fermentation.

Table 5. Pearson correlation matrix showing relationships between pigments (betacyanins βC , betaxanthins βX) and colorimetric parameters (CIELab).

	βC (mg L ⁻¹)	βX (mg L ⁻¹)	a*	b*	c*	L*	h*
βC (mg L ⁻¹)	1.00	7.9×10^{-7}	3.4×10^{-4}	0.03	0.01	1.7×10^{-3}	0.44
βX (mg L ⁻¹)	0.89	1.00	1.1×10^{-4}	4.3×10^{-5}	7.0×10^{-6}	2.3×10^{-6}	0.02
a*	0.75	0.79	1.00	3.8×10^{-4}	2.3×10^{-6}	4.4×10^{-7}	0.03
b*	0.52	0.81	0.75	1.00	5.1×10^{-12}	1.5×10^{-7}	5.8×10^{-8}
c*	0.63	0.85	0.87	0.98	1.00	3.0×10^{-10}	1.1×10^{-5}
L*	-0.68	-0.87	-0.90	-0.91	-0.96	1.00	6.0×10^{-4}
h*	0.19	0.56	0.52	0.92	0.84	-0.73	1.00

a*: red coordinate, b*: yellow coordinate, c*: chroma; L*: lightness, h*: hue angle, βC : betacyanin concentration (mg/L), βX : betaxanthin concentration (mg/L). Pearson's correlation coefficients (r) are shown below the main diagonal, while significance values (p) are presented above it. Correlations are classified as strong ($|r| \geq 0.70$), moderate ($0.40 \leq |r| < 0.70$), or low ($0.10 \leq |r| < 0.40$), with a positive direction when $r > 0$ and inverse when $r < 0$. Associations are considered significant at $p < 0.05$.

Conclusions

This study demonstrated that the carbon source plays a decisive role in the fermentation performance and functional properties of pitaya (*Stenocereus* sp.) wine. Among the evaluated formulations, agave syrup was the most suitable carbon source, promoting greater sugar utilization, ethanol production, fermentation yield, and fermentation efficiency than brown cane sugar. The formulation consisting of pitaya juice supplemented with agave syrup achieved the highest fermentation efficiency (89.71%) and an ethanol concentration of 84.16 g L^{-1} (approximately 10.7% v/v), indicating efficient conversion of fermentable sugars into ethanol.

Fermentation also influenced the functional characteristics of the beverages, and the formulation prepared with pitaya juice and agave syrup retained the highest antioxidant capacity after fermentation while maintaining elevated phenolic content. These results demonstrate that agave syrup represents a suitable alternative to brown cane sugar as a carbon source for pitaya wine production without compromising fermentation performance or functional quality. The proposed formulation represents a promising strategy for producing pitaya wine with improved fermentative performance and preserved functional properties.

Future studies should evaluate the physicochemical, microbiological, and functional stability of the selected formulation during storage to establish its shelf life and commercial applicability.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

Not applicable.

AUTHOR CONTRIBUTIONS

Conceptualization, S.R.S.-G.; L.S.-F., and J.A.S.-U.; methodology, L.S.-F.; S.R.S.-G.; J.A.S.-U., and R.G.O.-M.; software, R.G.O.-M., and O.G.-A.; validation, R.G.O.-M.; S.R.S.-G., and L.S.-F.; formal analysis, R.G.O.-M.; O.G.-A.; L.S.-F., and S.R.S.-G.; investigation, S.R.S.-G., and O. G.-A.; resources, S.R.S.-G.; data curation, L.S.-F., and R.G.O.-M; writing—original draft preparation, S.R.S.-G.; writing—review and editing, S.R.S.-G., J.A.S.-U.; visualization, S.R.S.-G.; supervision, S.R.S.-G.; project administration, S.R.S.-G.

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