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Anti-inflammatory, antioxidant, and phytochemical profiles of pineapple (*Ananas comosus* L. var. MD2) juice and wine produced through *Saccharomyces cerevisiae* (Desm.) Meyen batch fermentation

Walter Clint E. Bayani[1,*], Zeus S. Elumba[2], Lalaine Grace M. Robles[2] and Reggie Y. Dela Cruz[2]

[1]Graduate Student, Molecular Biology and Biotechnology, Genetics, and Microbiology (MBBGM) Division, Institute of Biological Sciences, College of Arts and Sciences, Central Mindanao University, University Town, Musuan, Bukidnon 8710 the Philippines
[2]MBBGM Division, Institute of Biological Sciences, College of Arts and Sciences, Central Mindanao University, University Town, Musuan, Bukidnon 8710 the Philippines

**Corresponding Author: clintbayani@gmail.com*

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ABSTRACT

This study examined the anti-inflammatory and antioxidant activities, total phenolic content (TPC), and high-performance thin-layer chromatography (HPTLC) profile of fresh pineapple (*Ananas comosus* L. var. MD2) juice, wine, and aged wine produced through *Saccharomyces cerevisiae* (Desm.) Meyen batch fermentation to evaluate their nutraceutical potential. Pineapple wine was produced by fermenting pasteurized juice at 20˚, 25˚, and 30˚ Brix under anaerobic conditions for three weeks, followed by a one-month maturation period for aged wine. Anti-inflammatory activity, total antioxidant capacity (TAC), and TPC were determined using the fluorescence cyclooxygenase (COX) inhibition assay, phosphomolybdenum method, and Folin–Ciocalteu assay, respectively. The HPTLC analysis was performed using a Chemie-Erzeugnisse und Adsorptionstechnik Muttenz AG (CAMAG) system, and consumer acceptability was assessed using a 9-point hedonic scale. Fermentation reduced juice acidity by 4–17% and yielded alcohol concentrations ranging from 5.17–11.8%. Both juice and wine inhibited COX-1 and COX-2 by over 50%, indicating significant anti-inflammatory activity. The TAC decreased by 32% from juice (2241.03 ± 55.22 mg AAE/L) to wine (1527.82 ± 92.52 mg AAE/L), and by 37% to aged wine (1416.03 ± 12.70 mg AAE/L). Similarly, TPC declined by 2% from juice (430.10 ± 5.08 mg GAE/L) to wine (422.57 ± 5.95 mg GAE/L) and by 19% to aged wine (348.30 ± 5.92 mg GAE/L). The HPTLC analysis revealed distinct chromatographic profiles in wine and aged wine, indicating the formation of metabolites. Among the aged wines, the 30˚ Brix sample received the highest sensory score (7.75). Therefore, fermentation preserves pineapple bioactivity and may enhance its nutraceutical value through the generation of unique metabolites.

Keywords: 9-point hedonic scale, bioactive compounds, cyclooxygenase inhibition, nutraceutical potential, phytochemicals

INTRODUCTION

Pineapple (*Ananas comosus* L.) is a highly valued tropical fruit and the most economically important species of the Bromeliaceae family. It is widely consumed in various forms, including fresh, cooked, juiced, or preserved (Adebayo-Tayo and Akpeji 2016). Known for its pleasant aroma and flavor,

pineapple is also a rich source of phytochemicals, dietary fiber, and essential nutrients, such as vitamins C, B1, and B6, and minerals including magnesium, calcium, and copper (Palachum et al. 2021). Pineapple also stands out for its remarkable health benefits, particularly its potent anti-inflammatory and antioxidant properties (Joy 2015). Bromelain, a proteolytic enzyme complex found predominantly in pineapple stems and juice, plays a pivotal role in modulating inflammation and has been extensively studied for its therapeutic effects (Varilla et al. 2021). Regular consumption of pineapple has also been shown to reduce hypercholesterolemia-induced cardiac lipid peroxidation and suppress pro-inflammatory responses *in vivo* (Seenak et al. 2021).

The Philippines is the world's second-largest pineapple producer after Costa Rica, with an estimated total export of 692,365 MT in 2024 (Business World 2025). Northern Mindanao led pineapple production in the Philippines in 2023, contributing 391.16 thousand MT to the country's production (PSA 2023). However, like many other fruits, pineapples are highly perishable due to their high moisture and nutrient content, which lead to significant post-harvest losses ranging from 20% to 50% (Kasso and Bekele 2018). The average post-harvest loss for pineapples in the Philippines is among the highest among agricultural products, at 30-40% (Mopera 2016). These losses can occur at various stages, including on-farm handling, transportation, storage, retail, processing, and at the consumer level. Therefore, preservation methods are considered essential to reduce post-harvest losses in the pineapple industry. Fermentation has been an effective method for food and beverage preservation for millennia (Ross et al. 2002). Fermenting fruit juices, such as pineapple, not only preserves the fruit but also increases its value by transforming it into new products like wine, which has a longer shelf life (Boondaeng et al. 2021).

Although pineapple is ideal for fermentation (Chanprasartsuk et al. 2010), and there is growing interest in pineapple wine production for local and export markets, limited research has examined its anti-inflammatory activity and high-performance thin-layer chromatography (HPTLC) profile. Previous studies mainly focused on its antioxidant properties and phenolic composition in fresh fruit or juice (Adeboyejo et al. 2018; Candrawinata et al. 2012), but little is known about whether fermentation and aging alter these bioactivities. Additionally, wine fermentation might concentrate or produce new metabolites that enhance its nutraceutical potential, creating an opportunity to set pineapple wine apart from other fruit wines. Such discoveries could increase the market appeal of pineapple wine, and help reduce post-harvest losses while enhancing the competitiveness of the Philippine pineapple industry.

This study aimed to examine the physicochemical properties (pH and total soluble solids), anti-inflammatory activity, total antioxidant capacity, total phenolic content, and HPTLC profile of fresh pineapple (*A. comosus* L. var. MD2) juice, wine, and aged wine produced through *S. cerevisiae* (Desm.) Meyen batch fermentation. It also sought to assess the consumer acceptability of the aged pineapple wine. Overall, the study provided a comprehensive evaluation of the biochemical properties, phytochemical profile, and potential health benefits of pineapple wine produced via *S. cerevisiae* batch fermentation.

METHODS

Pineapple Juice Extraction and Preparation

The fully ripe pineapple fruits (*A. comosus* var. MD2) were obtained from Wao, Lanao del Sur. Fruits were selected based on uniform size, yellow external peel color, and high sweetness aroma as indicators of ripeness. This ensured the homogeneity of the samples and minimized variability due to the maturity stage. The fruits were then washed with running tap water, peeled, and sliced into pieces. The juice was extracted using a high-quality kitchen juicer (Koii KMK-168) and filtered through filter paper to remove solid particles. It was then transferred to glass bottles with water valves to serve as fermentation containers. The total soluble solids (TSS) of the extracted juice were measured using a refractometer. To standardize the sugar content, granulated white sugar was added to adjust the TSS to fixed levels of 20˚, 25˚, and 30˚ Brix. This standardization allowed for controlled comparison of fermentation performance and product quality across treatments. Finally, the juice was pasteurized at 83˚C for 3 min and allowed to cool to approximately 50˚C before the addition of the yeast culture (Qi et al. 2017).

Wine Fermentation Set-up

A total of 2.5 g of active dry yeast (*S. cerevisiae*) was added to 500 mL of the pasteurized juice to initiate the fermentation. The mixture was stirred thoroughly. A control setup in which the juice underwent the same preparation steps, including sugar content adjustments, but without the addition of yeast, was also prepared. Fermentation was carried out for three weeks under anaerobic conditions using a fermentation valve to allow gas release while preventing air entry. After 3 weeks, the sediments were removed, and the supernatant was transferred to another bottle. Fermentation was stopped using sodium metabisulfite (1.32 mL/L wine). The resulting wine was then aged for one month.

Physicochemical Analysis (pH and total soluble solids)

The physicochemical parameters, such as pH and TSS of fresh juice, wine, and aged wine, were determined using a digital pH meter (HANNA HI6221) and a hand refractometer (THE01502), respectively. The alcohol content of the aged wine was determined using an ebulliometer (LDS Ref. 160250D). The alcohol contents of the 20˚ and 25˚ Brix control samples were not measured due to the limited sample volume after processing. Monitoring pH, TSS, and alcohol content provided key indicators of fermentation progress, acidity changes, and ethanol yield, which directly affected product quality and stability.

Determination of Anti-Inflammatory Activity of Fresh Pineapple Juice and Wine

The assay followed the methodology described by Bonner and Fry (2012). It was chosen to evaluate the anti-inflammatory potential of pineapple juice and wines by measuring their ability to inhibit cyclooxygenase enzymes, especially cyclooxygenase (COX)-2, which is a key enzyme involved in inflammation. First, 5,184 μL of 100 mM Tris buffer (pH 8) was transferred to a clean vial, followed by the addition of 96 μL of COX-2 and COX-1 enzymes (250 *u*/mL each) and 480 μL of 20 μM hemin to prepare the enzyme-cofactor solution. Next, 120 μL of this enzyme-cofactor solution was pipetted into each well of a 96-well microplate prefilled with 50 μL of 100 mM Tris buffer. To initiate the reaction, 10 μL of the test samples (diluted in ethanol to final concentrations of 50 mL/L and 100 mL/L) were added to the wells. Indomethacin (4 mM in 100% DMSO) served as a positive control. The plates were incubated at 25˚C for 15 min, after which 10 μL of 200 μM Amplex Red and 10 μL of 2000 μM arachidonic acid were added to each well. The solutions were mixed thoroughly and purged with nitrogen gas to remove oxygen. Fluorescence readings were taken every 12 s for 3 min using a CLARIOstar® microplate reader (BMG LABTECH) set at an excitation wavelength of 535 nm and an emission wavelength of 590 nm. The COX inhibitory activity was quantified based on the relative change in the slope of fluorescence intensity over time.

The positive control and the percentage inhibition of the samples were calculated using the average slope of each replicate using the following formula:

$$\% \text{ Inhibitory Activity} = \frac{Slope\ uninhibited - Slope\ inhibited}{Slope\ unhinhibited} \text{ x } 100\%$$

where $Slope_{uninhibited}$ represents the slope of the fluorescence vs. time plot for the negative control group, while $Slope_{inhibited}$ refers to the slope of the fluorescence vs. time plot for the samples or positive control (Ang et al. 2022).

Determination of Total Antioxidant Capacity (TAC) of Fresh Pineapple Juice, Wine, and Aged Wine

The TAC of the pineapple juice, wine, and aged wine samples was evaluated using the phosphomolybdenum method, following the procedure described by Prieto et al. (1999). This method was used as a broad measure of overall antioxidant capacity, indicating the samples' ability to donate electrons and potentially reduce oxidative stress. Briefly, 100 μL of test samples (diluted in ethanol to a final concentration of 50 mL/L) were mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95ºC for 90 min. Then, 200 μL of the resulting solutions were transferred to the designated microplate wells in quadruplicate. The absorbance of the solutions was measured at 695 nm using a Spectramax 250 Microplate Reader against a blank after cooling to room temperature. The antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per liter (mg AAE/L), based on a calibration curve generated using ascorbic acid.

Quantification of Total Phenolic Content (TPC) of Fresh Pineapple Juice, Wine, and Aged Wine

The TPC of the pineapple juice, wine, and aged wine samples was quantified using the Folin–Ciocalteu colorimetric method described by Ainsworth and Gillespie (2007). Phenolics are major contributors to antioxidant and anti-inflammatory effects in fruits and wines; therefore, quantifying TPC provides a direct measure of their nutraceutical potential. Briefly, 200 μL test samples (125 mL/L) were mixed with 200 μL of 10% of Folin-Ciocalteu reagent in a microcentrifuge tube and allowed to stand for 5 min. Subsequently, 800 μL of 10% sodium carbonate was added, and the mixture was incubated at room temperature for 30 min. The reaction mixture was centrifuged at 11,000 rpm for 3 min. Then, 200 μL of the resulting solution was transferred to the designated microplate wells in quadruplicate. The absorbance was measured at 750 nm using a Spectramax 250 Microplate Reader. The TPC was expressed as milligrams of gallic acid equivalents per liter (mg GAE/L), based on a calibration curve generated using gallic acid.

HPTLC Analysis of Fresh Pineapple Juice, Wine, and Aged Wine

An HPTLC system (CAMAG or Chemie-Erzeugnisse und Adsorptionstechnik Muttenz AG, Switzerland), equipped with an automatic TLC Sampler ATS 4, Automatic Developing Chamber ADC 2, Scanner 4, TLC Visualizer, Immersion Device 3, Plate Heater, and visionCATS 2.5 software, was used for analysis. Four (4) μL of test samples were applied onto an HPTLC aluminum-backed plate (silica

gel 60 F254; dimensions 20 × 10 cm; sourced from Merck) under a stream of nitrogen. Following application, the plate was developed in a pre-saturated twin-trough glass chamber (20 × 10 cm) maintained at a relative humidity of 33%. The mobile phase consisted of ethyl acetate, formic acid, and water (80:10:10). The chromatogram was visualized under both white light and UV light at wavelengths of 254 nm and 366 nm. Subsequently, the plate was immersed in a natural product (NP) reagent (2-aminoethyl diphenylborinate) (immersion speed: 5 cm/s; dwell time: 1 s), air-dried for 5 min under a fume hood, and visualized again under UV light at 366 nm (Ang et al. 2022; Jug et al. 2018). The HPTLC profiling enabled the detection and tentative identification of phenolic compounds, providing insight into the compositional changes and formation of novel metabolites during fermentation and aging.

Sensory Evaluation of Aged Pineapple Wine

The sensory evaluation for the aged wine was conducted in triplicate by a panel of 15 randomly selected and trained Bachelor of Science in Food Technology graduates from Central Mindanao University. The evaluation assessed the descriptors and acceptability of attributes, including color, aroma, taste, and overall acceptability of the wine (Boondaeng et al. 2021), utilizing a 9-point hedonic scale as follows: 1 - dislike extremely, 2 - dislike very much, 3 - dislike moderately, 4 - dislike slightly, 5 - neither like nor dislike, 6 - like slightly, 7 - like moderately, 8 - like very much, and 9 - extremely like.

Statistical Analysis

The effects of fermentation on TSS over time were analyzed using a two-way analysis of variance (ANOVA) to evaluate the main effects of time and Brix levels and their interaction. A mixed-effects model (Type III Wald F tests) with the Kenward-Roger method was used to improve the accuracy of F statistics and p-values (Kuznetsova et al. 2017). Prior to ANOVA, the assumptions of normality were evaluated using quantile–quantile (Q–Q) plots for graphical inspection and the Shapiro–Wilk test for statistical assessment.

A two-way ANOVA was also used for pairwise comparisons to assess differences between treatment groups. Additionally, a Kruskal-Wallis test was conducted ($P < 0.05$) to evaluate overall group differences (Nwiyi et al. 2023). For multiple comparisons, Tukey's honest significant difference (HSD) test and the Bonferroni correction were applied to adjust for multiple testing and ensure statistical robustness (Cosme et al. 2024; Ruppert et al. 2021). All statistical analyses were performed using R software, with statistical significance set at $P < 0.05$ and additional significance thresholds ($P < 0.001$, $P < 0.05$).

These statistical approaches were applied to ensure a robust evaluation of the main effects, interactions, and group differences while accounting for assumption checks and controlling Type I error.

RESULTS

Physicochemical Properties (pH and Total Soluble Solids)

The fresh pineapple juice exhibited a pH of 3.94 ± 00 and a TSS of 15˚ Brix. After 21 days of fermentation, the pH of the resulting pineapple wine slightly decreased, indicating increased acidity. The final pH of the control and treated samples at 20˚ Brix were 3.70 ± 00 and 3.73 ± 0.03, respectively. For the 25˚ Brix samples, the pH decreased to 3.75 ± 00 (control) and 3.77 ± 0.01 (treated). At 30˚ Brix, the control had a final pH of 3.28 ± 00, while the treated sample had a pH of 3.73 ± 0.03. The Total Soluble Solids (TSS) decreased after fermentation (Figure 1). The juices with 20˚ Brix and 25˚ Brix decreased to 7 ± 0.00˚ Brix and 9 ± 0.00˚ Brix, respectively. Meanwhile, the juices with 30˚ Brix showed a final TSS values of 21.6 ± 0.00˚ Brix for the control and 14˚ Brix for the wine (Table 1). These findings indicate that fermentation reduced the TSS (i.e., sugar concentration) of the juice, leading to the formation of alcohol.

The statistical analysis using a mixed-effects model (Type III Wald F tests with Kenward-Roger degrees of freedom) revealed significant effects of fermentation time and Brix levels on the TSS of the juice (Table 2). The main factors – Week to Month (time), Brix concentration, and juice type - all had highly significant effects ($P < 0.001$), confirming that these variables significantly influenced TSS reduction. Furthermore, the interactions between week to month × Brix, week to month × juice, and Brix × juice were also highly significant ($P < 0.001$), indicating that the effect of fermentation on TSS depended on both initial sugar concentration (Brix) and juice type. The three-way interaction week to month × Brix × juice was also highly significant ($P < 0.001$), suggesting that the combined effects of fermentation duration, initial sugar concentration, and juice type played a crucial role in TSS reduction.

The alcohol content of the wines ranged from 5.17 ± 0.12% to 11.8 ± 0.00%. The wine produced from 30˚ Brix juice exhibited the highest alcohol concentration at 11.8 ± 0.00%, while the lowest was observed in the wine produced from 20˚ Brix juice, with 7.17 ± 0.06%. The wine from the control group (30˚ Brix juice) had an alcohol content of 5.17 ± 0.12% (Figure 2). The alcohol content for the wines produced from 20˚ and 25˚ Brix juice control groups was not determined due to limited sample volume after processing.

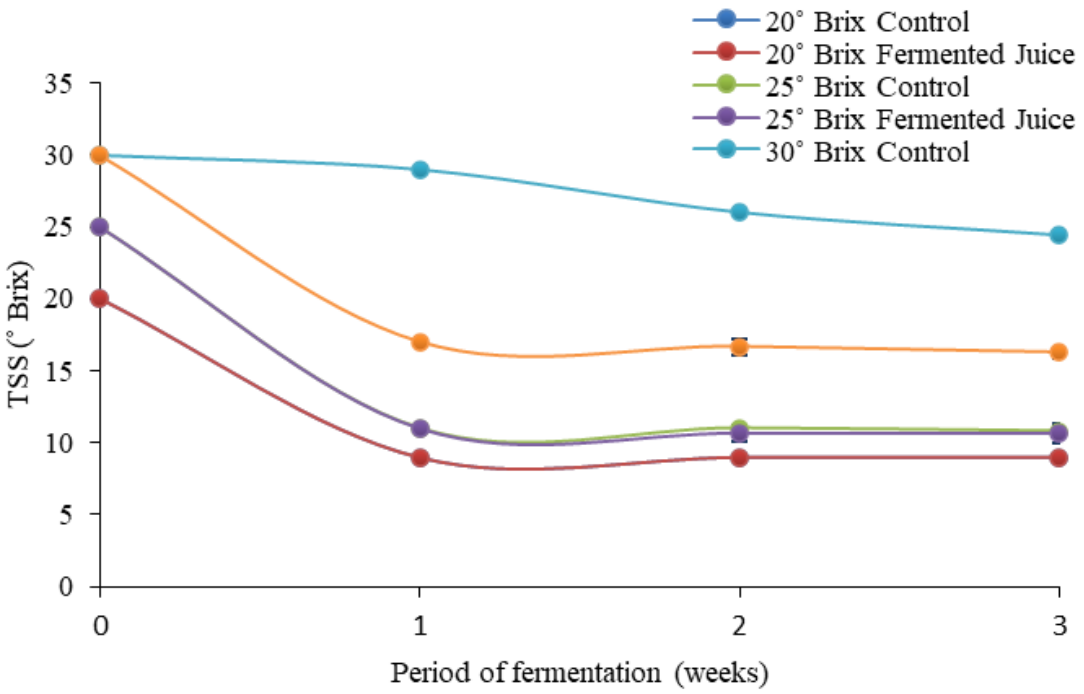

Figure 1. Effect of fermentation period, levels of initial total soluble solids on the rate of fermentation.

Table 1. Initial and final pH, total soluble solids (TSS) and alcohol content of juice and the resulting pineapple wine after aging (mean ± SD, n = 3).

Sample	pH		TSS (° Brix)	
	Initial (n = 3.94 ± 00)	Final	Initial	Final (Aged)
20° Brix Control	3.94 ± 00	3.70 ± 00	20 ± 0.00	7 ± 0.00
20° Brix	3.94 ± 00	3.73 ± 0.03	20 ± 0.00	7 ± 0.00
25° Brix Control	3.94 ± 00	3.75 ± 00	25 ± 0.00	9 ± 0.00
25° Brix	3.94 ± 00	3.77 ± 0.01	25 ± 0.00	9 ± 0.00
30° Brix Control	3.94 ± 00	3.28 ± 00	30 ± 0.00	21.60 ± 0.00
30° Brix	3.94 ± 00	3.73 ± 0.03	30 ± 0.00	14 ± 0.20

Table 2. Analysis of deviance table for mixed effects model (Type III Wald F Tests with Kenward-Roger df) on total soluble solids. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Factor	F Value	Df	Df. Residual	*P*-value
Week to Month	884.47	3	36	< 2.22e-16***
Brix	278.71	2	12	8.76E-11***
Juice	184.35	1	12	1.21E-08***
Week to Month: Brix	215.19	6	36	< 2.22e-16***
Week to Month: Juice	265.92	3	36	< 2.22e-16***
Brix: Juice	985.1	2	12	4.92E-14***
Week to Month: Brix: Juice	168.58	6	36	< 2.22e-16***

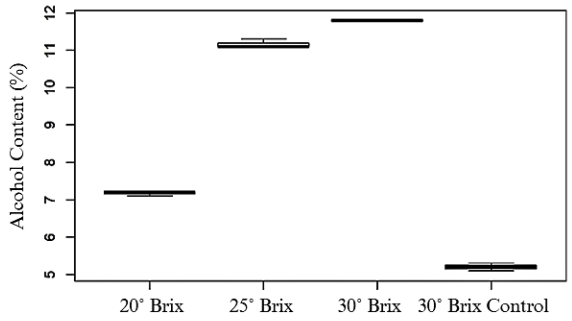

Figure 2. Alcohol content of the resulting aged pineapple wines. Values represent the mean of triplicate fermentations.

A Kruskal-Wallis test, a non-parametric alternative to ANOVA, was conducted to evaluate differences in alcohol content among the Brix levels (20˚, 25˚, 30˚, and 30˚ control). The test indicated a statistically significant difference among the groups (χ^2 = 10.607, df = 3, *P* = 0.01405), suggesting that alcohol content varied with the initial sugar concentration. Pairwise comparisons revealed that only the 30˚ Brix wine and the 30˚ Brix control differed significantly (*P* = 0.0060), demonstrating that fermentation, rather than sugar content alone, was a key factor in determining the final alcohol content at this sugar level compared with the non-fermented control (Table 3).

Table 3. Kruskal-Wallis rank sum test and post hoc Bonferroni comparison of alcohol content of aged pineapple wines. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Comparison	Value	*P*-value
Kruskal-Wallis	χ^2= 10.607	0.01405
Group Comparison	**Z-scores**	***P*-value**
25˚ Brix vs. 20 Brix	-1.0299s	0.9092
30˚ Brix vs. 20 Brix	-2.0598	0.1182
30˚ Brix vs. 25 Brix	-1.0299	0.9092
30˚ Brix vs. 20 Brix	1.0299	0.9092
30˚ Brix vs. 25 Brix	2.0598	0.1182
30˚ Brix vs. 30 Brix	3.0897	0.0060*

Anti-inflammatory Activity of Fresh Pineapple Juice and Wine

Fresh pineapple juice and wine, both produced from 30˚ Brix juice fermented with *S. cerevisiae*, were tested for their inhibitory effects on COX-1 and COX-2 enzymes at concentrations of 50 mL/L and 100 mL/L. The results showed that fresh pineapple juice inhibited COX-2 by 76.73% ± 2.49 at 50 mL/L and 84.53 ± 0.28% at 100 mL/L, while the wine exhibited inhibition rates of 79.77 ± 1.42% and 80.44 ± 1.83%, respectively (Table 4). Notably, at the higher concentration of 100 mL/L, fresh pineapple juice demonstrated greater COX-2 inhibitory activity than the standard drug indomethacin, which showed a COX-2 inhibition rate of 83.90 ± 3.22%. For COX-1 inhibition, fresh juice showed inhibition rates of 61.72 ± 9.45% and 76.33 ± 1.26% at 50 mL/L and 100 mL/L, respectively, while the wine exhibited lower inhibition rates of 49.72 ± 0.25% and 74.31 ± 1.14% (Figure 3).

A two-way ANOVA was performed to examine the effects of COX enzyme type (COX-1 and COX-2) and concentration on inhibition rates. The analysis revealed statistically significant main effects for both COX type (F = 104.44, *P* = 2.19e-09) and concentration (F = 28.36, *P* = 5.43e-08), indicating that each factor significantly affected enzyme inhibition. More importantly, a significant interaction effect was observed between COX type and concentration (F = 14.28, *P* = 1.16e-05) (Table 5). This interaction indicates that the inhibitory effects of fresh juice and wine on COX enzymes varied across concentrations, meaning that the effect of one factor depended on the level of the other.

Pairwise comparisons using Tukey's honestly significant difference (HSD) test revealed significant differences in COX inhibition among treatments. Fresh pineapple juice at 100 mL/L showed significantly higher COX-1 inhibition compared with that at 50 mL/L (*P* = 0.0003), while wine at 100 mL/L demonstrated significantly greater COX-1 inhibition than wine at 50 mL/L (*P* < 0.0001). Fresh juice at 50 mL/L showed significantly lower COX-1 inhibition than indomethacin (*P* < 0.0001), indicating that although pineapple juice exhibited anti-inflammatory potential, its COX-1 inhibitory activity at lower concentrations was weaker than that of the standard drug. However, the higher COX-1 inhibition observed in wine at higher concentrations suggested that fermentation may increase or preserve certain bioactive compounds responsible for COX-1 inhibition, thereby enhancing its effectiveness at higher doses. For COX-2 inhibition, no significant differences were found among most treatments (*P* > 0.05), except for fresh juice at 100 mL/L, which showed slightly higher inhibition than fresh juice at 50 mL/L (*P* = 0.0727) (Table 6). The absence of statistical significance in COX-2 inhibition suggests that both fresh juice and wine maintained similar potency across different concentrations, with fermentation likely helping to preserve their anti-inflammatory activity.

Table 4. COX-2 and COX-1% inhibition of fresh juice (50 mL/L and 100 mL/L) and 30˚ Brix pineapple wine (50 mL/L and 100 mL/L).

Sample	Concentration	COX-2 (%)	COX-1 (%)	COX-2: COX-1
Indomethacin	4 mM	83.90 ± 3.22	79.70 ± 1.37	1.05
Fresh Juice	50 mL/L	76.73 ± 2.49	61.72 ± 9.45	1.24
Wine	50 mL/L	79.77 ± 1.42	49.72 ± 0.25	1.60
Fresh Juice	100 mL/L	84.53 ± 0.28	76.33 ± 1.26	1.11
Wine	100 mL/L	80.44 ± 1.83	74.31 ± 1.14	1.08

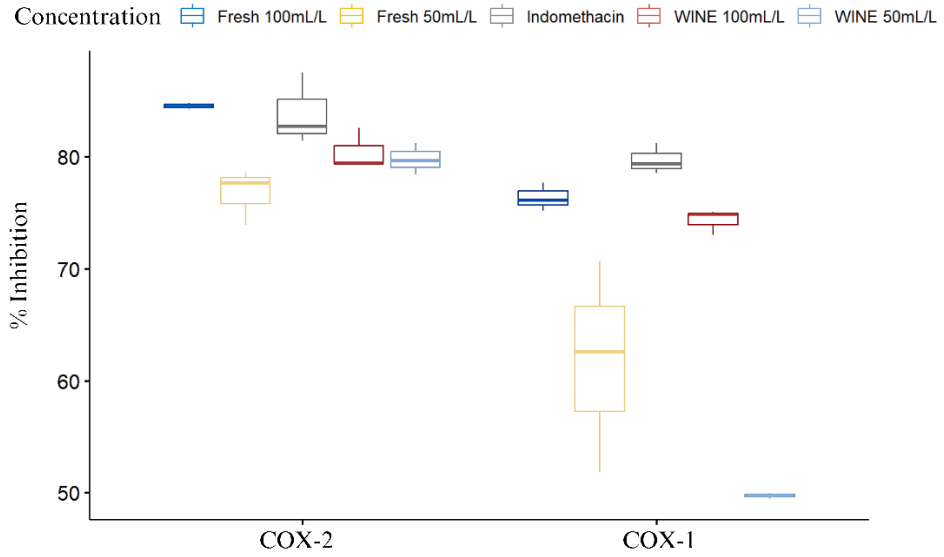

Figure 3. Boxplot showing the inhibitory activity of the fresh juice and wine from 30˚ Brix juice against COX-1 and COX-2.

Table 5. Two-way ANOVA results showing the significant effects of COX type, concentration, and their interaction on inhibition rates. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Source of Variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)
COX	1	1212.6	1212.6	104.44	2.19e-09 ***
Concentration	4	1317.3	329.3	28.36	5.43e-08 ***
COX: Concentration	4	663.1	165.8	14.28	1.16e-05 ***
Residuals	20	232.2	11.6		

Table 6. Pairwise comparisons of COX-1 and COX-2 inhibition. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Contrast	COX-1 (*P*-value)	COX-2 (*P*-value)
Fresh 100 mL/L - Fresh 50 mL/L	0.0003^{***}	0.0727^{ns}
Fresh 100 mL/L - Indomethacin	0.7453^{ns}	0.9993^{ns}
Fresh 100 mL/L - Wine 100 mL/L	0.9476^{ns}	0.5904^{ns}
Fresh 100 mL/L - Wine 50 mL/L	0.0004^{***}	0.4485^{ns}
Fresh 50 mL/L - Indomethacin	$<0.0001^{***}$	0.1343^{ns}
Fresh 50 mL/L - Wine 100 mL/L	0.0017^{**}	0.6774^{ns}
Fresh 50 mL/L - Wine 50 mL/L	0.0028^{**}	0.8088^{ns}
Indomethacin - Wine 100 mL/L	0.3304^{ns}	0.5836^{ns}
Indomethacin - Wine 50 mL/L	$<0.0001^{***}$	0.7480^{ns}
Wine 100 mL/L - Wine 50 mL/L	$<0.0001^{***}$	0.9002^{ns}

Both fresh juice and wine showed selectivity for COX-2 over COX-1, with COX-2/COX-1 inhibition ratios greater than 1.0 (1.08 - 1.60). These results indicate strong COX-2 inhibitory effects of pineapple juice and wine, with fresh juice showing more potent activity at higher concentrations. The fermentation process appeared to maintain the anti-inflammatory properties of pineapple, although small variations in COX enzyme inhibition were observed.

Total Antioxidant Capacity (TAC) and Total Phenolic Content (TPC) of Fresh Pineapple Juice, Wine, and Aged Wine

The TAC of fresh pineapple juice was notably higher (2241.03 ± 55.22 mg AAE/L) compared with wine (1527.82 ± 92.52 mg AAE/L) and aged wine (1416.03 ± 12.70 mg AAE/L) (Table 7; Figure 4). A Kruskal-Wallis test revealed a significant difference among the groups ($\chi^2 = 6.25$, $P = 0.04$). Post hoc analysis using the Bonferroni correction showed that the TAC of fresh juice was significantly higher than that of aged wine ($P = 0.0203$). Meanwhile, no significant differences were observed between fresh juice and wine ($P = 0.1740$) or between wine and aged wine ($P = 0.5536$) (Table 8; Figure 5).

Similarly, the TPC of fresh juice was the highest (430.10 ± 5.08 mg GAE/L), compared with wine (422.57 ± 5.95 mg GAE/L) and aged wine (348.30 ± 5.92 mg GAE/L) (Table 7). A Kruskal-Wallis test confirmed a significant difference among the groups ($\chi^2 = 6.4889$, $P = 0.03899$). Post hoc analysis with Bonferroni correction showed that fresh juice had significantly higher TPC than aged wine ($P = 0.0169$), while no significant differences were observed between fresh juice and wine ($P = 0.4451$) or between wine and aged wine ($P = 0.2041$) (Table 9).

HPTLC Profile of Fresh Pineapple Juice, Wine, and Aged Wine

The analysis was performed on pineapple juice, wine (30˚ Brix), and aged wine (30˚ Brix) to evaluate their phytochemical profiles. The HPTLC analysis of these samples revealed chromatographic profiles with 7, 10, and 10 blue fluorescent bands, respectively, under UV light at 366 nm. These bands were characterized by distinct Rf values, ranging from 0.02 to 0.83 for fresh juice, 0.03 to 0.84 for wine, and 0.02 to 0.84 for aged wine (Table 10). The blue fluorescence observed in these bands was commonly associated with phenolic acids. Additionally, the presence of these fluorescent bands in all samples suggests that phenolic compounds remained stable during fermentation and aging. However, differences in Rf values and band intensities indicated changes in the relative amounts of individual phytochemicals (Figure 6).

Sensory Evaluation of Aged Pineapple Wine

The average sensory evaluation scores of aged pineapple wines, assessed using a 9-point hedonic scale, are presented in Table 11. Among the samples, the wine produced from 30˚ Brix juice achieved the highest ratings across all attributes, including color (7.64 ± 0.04), aroma (7.31 ± 0.27), taste (7.73 ± 0.07), and overall acceptability (7.75 ± 0.08).

Table 7. Total antioxidant capacity (50mL/L) and total phenolic content (125mL/L) of fresh pineapple juice, wine and aged wine.

Samples	TAC (mg AAE/L)	TPC (mg GAE/L)
Fresh Juice	2241.03 ± 55.22	430.10 ± 5.08
Wine	1527.82 ± 92.52	422.57 ± 5.95
Aged Wine	1416.03 ± 12.70	348.30 ± 5. 92

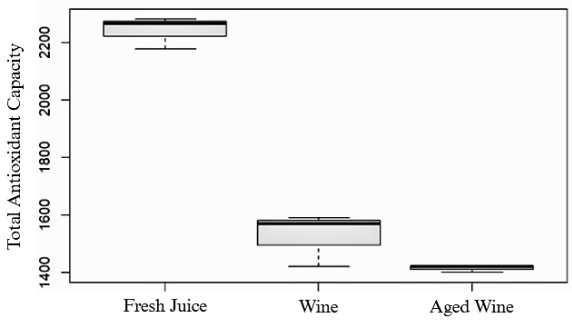

Figure 4. Boxplot showing the total antioxidant capacity (50mL/L) of fresh pineapple juice, wine and aged wine.

Table 8. Kruskal-Wallis test and post hoc pairwise comparisons of total antioxidant capacity among pineapple juice, wine, and aged wine. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Comparison	Value	*P*-value
Kruskal-Wallis Test	$\chi^2 = 6.2521$	0.04
Pairwise Comparisons	**Z-score**	***P*-value (adjusted)**
Aged Wine vs. Juice	-2.469987	0.0203*
Aged Wine vs. Wine	-0.898177	0.5536
Juice vs. Wine	1.57181	0.1739

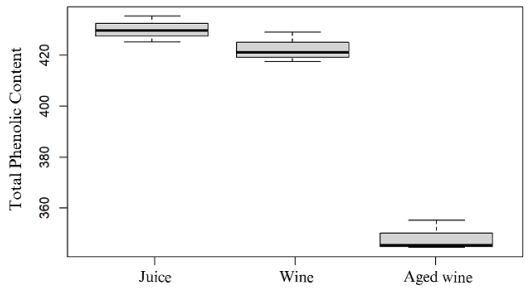

Figure 5. Boxplot showing the total phenolic content (125mL/L) of fresh pineapple juice, wine and aged wine.

Table 9. Kruskal-Wallis test and post hoc pairwise comparisons of total antioxidant capacity among pineapple juice, wine, and aged wine. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Comparison	**Value**	***P*-value**
Kruskal-Wallis Test	$\chi^2 = 6.4889$	0.04
Pairwise Comparisons (Bonferroni)	**Z-score**	***P*-value**
Juice vs. Aged Wine	-2.53421s	0.0169*
Wine vs. Aged Wine	-1.490711	0.2041
Juice vs. Wine	1.043498	0.4451

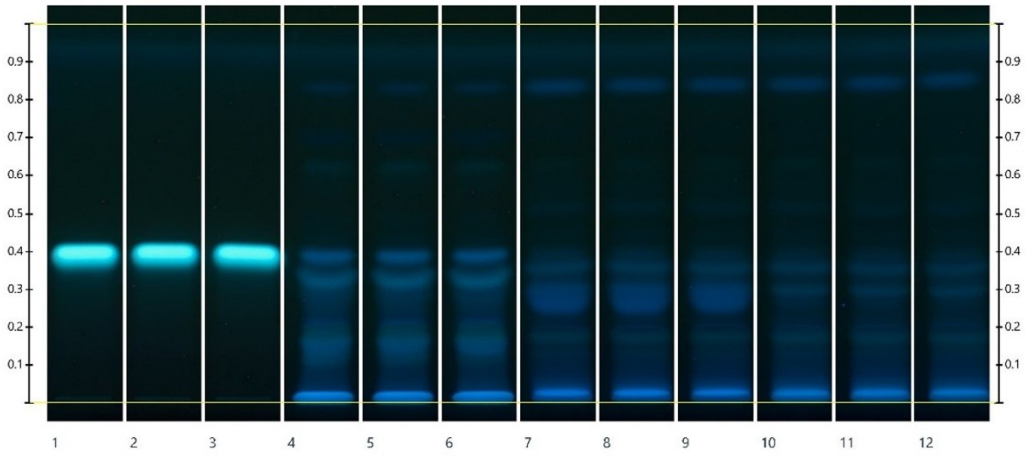

Figure 6. High-performance thin layer chromatography profiles of Chlorogenic acid (track 1-3), fresh pineapple juice (track 4-6), wine (track 7-9), and aged wine (track 10-12) visualized under ultraviolet light at 366 nm following elution with an ethyl acetate: formic acid: water (80:10:10) and derivatization using natural product reagent.

Table 10. High-performance thin layer chromatography profile of the fresh juice, wine and aged wine visualized under ultraviolet light at 366 nm ethyl acetate: formic acid: water (80:10:10) under 366 nm as mobile phase and natural product as a derivatizing agent.

Range of Rf Values	**Chlorogenic Acid**		**Fresh Juice**		**Wine**		**Aged Wine**	
	Rf	**Color**	**Rf**	**Color**	**Rf**	**Color**	**Rf**	**Color**
0.00-0.20			0.02	Blue	0.03	Blue	0.02	Blue
			0.16	Blue	0.17	Blue	0.17	Blue
0.21-0.40					0.26	Blue	0.27	Blue
					0.29	Blue	0.29	Blue
			0.32	Blue	0.36	Blue	0.35	Blue
	0.4	Blue	0.39	Blue	0.39	Blue	0.39	Blue
0.41-0.60					0.52	Blue	0.52	Blue
					0.57	Blue	0.57	Blue
0.61-0.80			0.62	Blue	0.63	Blue	0.64	Blue
			0.70	Blue				
0.80-11.00			0.83	Blue	0.84	Blue	0.84	Blue

The 30˚ Brix control wine received slightly lower but still favorable scores, with an overall acceptability of 7.47 ± 0.07, corresponding to the "liked moderately " to "liked very much" range. In contrast, wines from the 20˚ Brix control (6.93 ± 0.13), 20˚ Brix juice (6.91 ± 0.27), 25˚ Brix juice (6.88 ± 0.08), and 25˚ Brix control (6.80 ± 0.24) were rated lower, generally falling between "like slightly" and "like moderately." These results suggest that a higher initial sugar concentration (30˚ Brix) contributed positively to sensory attributes and consumer acceptability of aged pineapple wine. Furthermore, pineapple wine produced from 30˚ Brix juice exhibited the most favorable sensory qualities, particularly in taste and overall acceptability, making it more appealing to consumers than wines produced from lower Brix levels.

A two-way ANOVA revealed statistically significant effects of Brix level (F = 18.96, $P < 0.001$), acceptability factors (F = 8251.65, $P < 0.001$), and their interaction (F = 60.41, $P < 0.001$) on sensory evaluation scores. Simple main effects analysis indicated significant differences in aroma (F = 46.86, $P < 0.001$), color (F = 61.32, $P < 0.001$), and taste (F = 49.16, $P < 0.001$). Post hoc pairwise comparisons showed that color and taste differed significantly ($P < 0.001$), whereas aroma and taste did not differ significantly ($P = 0.1368$). In terms of descriptive analysis, aroma-color ($P < 0.001$), aroma-taste ($P < 0.001$), and color-taste ($P < 0.001$) all displayed significant differences (Table 12). These results suggest that the juice's sweetness level (measured by Brix) significantly influenced consumer perception of pineapple wine, with higher sugar content leading to improved color, taste, and overall sensory appeal.

Panelists' color descriptions ranged from 7.29 ± 0.10 to 7.64 ± 0.04, reflecting moderate acceptance. The hues were identified as shades between Buttered Rum (#A16D0A) and Muddy Brown (#945C06). Bitterness was noted in wines from 20˚ Brix and 25˚ Brix juices and their corresponding controls, while wines from 30˚ Brix juices exhibited a balanced bittersweet to moderately sweet flavor profile.

This analysis confirmed that higher Brix levels (30˚ Brix) led to significantly higher acceptability scores compared to lower Brix levels (20˚ and 25˚ Brix), aligning with the preference for sweeter and more balanced flavor profiles in pineapple wine (Figure 7).

Table 11. Sensory evaluation of pineapple wine with various sugar level. Desc – Description, Acc – Acceptability.

Sample	Color		Aroma		Taste		Overall Acceptability
	Desc.	Acc.	Desc.	Acc.	Desc.	Acc.	
20˚ Brix Control	2.09 ± 0.28	7.44 ± 0.14	2.84 ± 0.32	7.28 ± 0.20	1.76 ± 0.21	6.64 ± 0.14	6.93 ± 0.13
20˚ Brix	2.02 ± 0.17	7.47 ± 0.18	2.76 ± 0.23	7.04 ± 0.21	1.87 ± 0.06	6.69 ± 0.43	6.91 ± 0.27
25˚ Brix Control	1.98 ± 0.25	7.49 ± 0.10	2.6 ± 0.18	6.98 ± 0.28	1.58 ± 0.10	6.56 ± 0.15	6.80 ± 0.24
25˚ Brix	1.40 ± 0.20	7.29 ± 0.10	2.71 ± 0.25	7.11 ± 0.10	1.93 ± 0.07	6.73 ± 0.13	6.88 ± 0.08
30˚ Brix Control	1.09 ± 0.08	7.36 ± 0.25	3.2 ± 0.37	7.27 ± 0.12	4.24 ± 0.20	7.58 ± 0.10	7.47 ± 0.07
30˚ Brix	1.82 ± 0.23	7.64 ± 0.04	2.69 ± 0.14	7.31 ± 0.27	3.07 ± 0.17	7.73 ± 0.07	7.75 ± 0.08

Table 12. Two-Way ANOVA and pairwise comparisons on sensory evaluation. $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$; ^{ns}-not significant.

Analysis	Factor(s)	F/t Value	*P*-Value
Two-Way ANOVA	Factors	18.96	7.27E-09***
	Acceptance	8251.65	< 2e-16***
	Factors × Acceptance	60.41	< 2e-16***
Simple Main Effects	Aroma	46.855	< 0.0001***
	Color	61.321	< 0.0001***
	Taste	49.16	< 0.0001***
Pairwise Comparisons (Acceptability)	Aroma - Color	-3.02	0.0072**
	Aroma - Taste	1.908	0.1368^{ns}
	Color - Taste	4.928	< 0.0001***
Pairwise Comparisons (Description)	Aroma - Color	11.446	< 0.0001***
	Aroma - Taste	4.213	0.0001***
	Color - Taste	-7.233	< 0.0001***

Figure 7. Fermented pineapple juice (20˚ Brix: 1-R1, 2-R2, 3-R3, 4-control; 25˚ Brix: 5-R1, 6-R2, 7-R3, 8-control; 30˚ Brix: 9-R1, 10-R2, 11-R3, 12-control).

DISCUSSION

Physicochemical Properties (pH and Total Soluble Solids)

The observed physicochemical changes in pineapple juice and wine are consistent with previous studies, supporting the use of pineapple juice as a substrate for winemaking. The decrease in pH across all samples, from an initial 3.94 ± 00 in fresh juice to a range of 3.28 ± 00 to 3.77 ± 0.01 in wines, corresponds to a decrease of approximately 0.17 to 0.66 pH units (equivalent to 4–17%), and aligns with the typical increase in acidity during fermentation (Boondaeng et al. 2021). This acidification is important for improving the wine's stability and flavor profile (Van Man 2021). The small variations in wine pH may be attributed to differences in yeast metabolic activity and the initial sugar content of the juice.

The total soluble solids (TSS) decreased significantly during fermentation, particularly in the first week, reflecting the rapid consumption of sugars by *S. cerevisiae*. This aligns with previous studies, which indicate that the initial stages of fermentation are the most active, with sugar levels dropping rapidly as glucose and fructose are converted into ethanol and carbon dioxide (Van Man 2021). The final stabilization of TSS values, usually around the second week, indicates the near completion of sugar consumption and marks the end of fermentation.

Interestingly, the TSS in the control group (no yeast) also declined, probably due to the activity of native yeasts and bacteria in the juice, such as *Pichia guilliermondii* Wickerham and *Hanseniaspora uvarum* (Niehaus) Shehata, Mrak & Phaff ex M.T. Sm. (Di Cagno et al. 2010; Chanprasartsuk et al. 2010). This underscores the potential role of native microbial flora in driving spontaneous fermentation processes, even without the addition of *S. cerevisiae*. Additionally, the presence of Firmicutes on pineapple peel, particularly the genera *Weissella, Lactobacillus,* and *Lactococcus* (Tallei et al. 2022), may have facilitated spontaneous fermentation in the control group.

The alcohol content of the wines ranged from 5.17 ± 0.12% to 11.80 ± 0.00%, with higher sugar content in the juice resulting in higher ethanol levels. This positive correlation between TSS and alcohol content is well-documented in many fermentation studies (Van Man 2021). Notably, the 30˚ Brix juice sample, which contained the highest sugar concentration, yielded the highest alcohol content (11.80 ± 0.00%), demonstrating the direct influence of sugar availability on ethanol production. The fermentation process, driven by *S. cerevisiae*, efficiently converted the sugars into ethanol, confirming the yeast's crucial role in alcohol yield.

The lower alcohol content in the 30˚ Brix juice no-yeast control group (5.17 ± 0.12%) compared to its yeast-inoculated counterpart (11.80 ± 0.00%) further highlights the importance of using a controlled yeast strain to achieve higher ethanol production. While natural fermentation can occur through native microbial activity, it is significantly less efficient at producing high alcohol concentrations without the addition of a selected yeast strain.

Anti-inflammatory Activity of Fresh Pineapple Juice and Wine

Both fresh pineapple juice and the resulting wine showed COX-2 selective inhibition of COX-2, indicating potential anti-inflammatory effects. The higher COX-2/COX-1 inhibition ratio suggests that

these samples specifically target COX-2 over COX-1, which is a desirable trait in anti-inflammatory agents, as COX-1 inhibition can cause adverse gastrointestinal effects (Radi and Khan 2006). In contrast, COX-2 is an inducible isoform mainly found in inflammatory cells and tissues in response to stimuli such as cytokines, mitogens, endotoxins, hormones, tumor promoters, carcinogens, wounding, and ultraviolet (UV) radiation (Jain et al. 2008; Rundhaug and Fischer 2011). The assay's reliability is supported by its consistency with the positive control, which usually shows 60-80% inhibition at 4 mM (Opog and Amor 2019).

Despite fermentation, which typically alters the chemical composition of the juice, the anti-inflammatory activity of pineapple juice was retained, indicating that key bioactive compounds responsible for COX-2 inhibition remained stable. The slight decrease in COX-2 and COX-1 inhibitory activities at higher concentrations (100 mL/L) may indicate a threshold effect, beyond which diminishing returns or a balancing effect on both COX enzymes may occur. The selective increase in COX-2 activity at lower concentrations (50 mL/L), along with the decrease in COX-1 inhibition, demonstrates a concentration-dependent effect on enzyme inhibition.

The anti-inflammatory effects observed in both fresh pineapple juice and wine may be attributed to bioactive phytochemicals that modulate key enzymatic pathways involved in inflammation. Hidaka et al. (2008) demonstrated that bromelain from pineapple can suppress the expression of CD25, a transmembrane protein usually upregulated in activated T cells, and can also inhibit cyclooxygenase-2 (COX-2) expression. These mechanisms support the potential anti-inflammatory and antitumor properties of bromelain (Bhui et al. 2009; Secor et al. 2009). In addition to these bioactive compounds, pineapple is a rich source of essential nutrients and vitamins, including ascorbic acid, folate (DFE), niacin, vitamin B6, riboflavin, thiamin, and vitamins A, E, and K. It also contains important minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc (USDA-NRCS 2014). These nutritional components further contribute to the wide range of health benefits associated with pineapple consumption.

Total Antioxidant Capacity and Total Phenolic Content of Fresh Pineapple Juice, Wine and Aged Wine

Free radicals can cause oxidative damage that may build up over time and potentially lead to degenerative diseases (Zubia and Dizon 2019). Antioxidants are substances capable of neutralizing free radicals. The observed decreases in TAC and TPC values may be attributed to various factors related to juice processing, including clarification, filtration, and pasteurization. These processes can remove phenolic compounds associated with dietary fiber and pectin in pineapple (Candrawinata et al. 2012). Furthermore, thermal processing has been shown to degrade anthocyanins in grapes (Kechinski et al. 2010).

Khalid et al. (2016) reported that pineapple juice inhibits the activity of cytochrome P450 2C9, an enzyme that plays a crucial role in the oxidation and metabolism of many therapeutic drugs. Additionally, pineapple contains the alkaloid 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-α-carboline, which exhibits antioxidant properties (Herraiz and Galisteo 2003). It is widely accepted that much of the antioxidant activity in pineapple juice is attributable to its phenolic compounds (Khalid et al. 2016). Li et al. (2014) identified the primary polyphenolics in pineapples as gallic acid, catechin, epicatechin, and ferulic acid. In addition, flavonoids such as catechin, epicatechin, and myricetin in pineapples are considered effective antioxidants because of the number and position of hydroxyl groups (Domínguez et al. 2018).

Previous phytochemical analyses of pineapple extracts revealed that the phenolic content, expressed as caffeic acid equivalents, was highest in the methanol extract, followed by the ethyl acetate extract and the water extract. The antioxidant capacity of these extracts, expressed in terms of ascorbic acid equivalents (μmol g^{-1} of extract), ranked in descending order: methanol extract, ethyl acetate extract, and water extract (Hossain and Rahman 2011). Adeboyejo et al. (2018) highlighted that pineapple cultivars grown in Nigeria are particularly rich in antioxidants, characterized by high levels of flavonoids, phenolic content, and overall antioxidant activity. In a related study, Boondaeng et al. (2021) reported an increase in phenolic content from the first to the last day of fermentation, accompanied by a decrease in antioxidant capacity. Overall, these findings revealed the potential of fresh pineapple juice and, to a lesser extent, pineapple wine, as valuable sources of natural antioxidants.

High-Performance Thin Layer Chromatography (HPTLC) Profile of Fresh Pineapple Juice, Wine and Aged Wine

Pineapple juice is a valuable commercial product; however, its phenolic profile largely remains uncharacterized (Khalid et al. 2016). To address this gap, HPTLC analysis was conducted on samples of pineapple juice, wine, and aged wine.

The presence of blue fluorescence observed in these bands was commonly attributed to phenolic acids (Bernardi et al. 2019; Ang et al. 2022). Further analysis showed that the Rf values for the test samples (0.39) closely matched those of the standard chlorogenic acid (0.40). Khan et al. (2020) and Samreen et al. (2020) demonstrated that pineapples contain chlorogenic acid. Therefore, it is likely that the

band at an Rf value of 0.39 corresponds to chlorogenic acid. However, confirmation of this identification using gas chromatography-mass spectrometry (GC-MS) is necessary to verify that the compound is indeed chlorogenic acid.

New bands appeared after fermentation, with an Rf value of 0.52 observed in both wine and aged wine. Procopio et al. (2013) showed that yeast fermentation breaks down glycosidic precursors, releasing bioactive compounds such as monoterpenoid alcohols, terpene oxides, and diols. This enzymatic activity is important in changing the chemical composition of fermented products. Similarly, during pineapple wine fermentation, yeast may have broken down glycosidic precursors in the juice, generating new metabolites that contributed to the observed bands. This biochemical change could explain the appearance of new bands in the chromatographic profile, indicating structural modifications or the formation of new metabolites. These findings suggest that fermentation not only improves the volatile content and sensory qualities of pineapple wine but also alters its phytochemical profile, potentially enhancing its functional properties.

Sensory Evaluation of Aged Pineapple Wine

Sensory evaluation of pineapple wines prepared from different pineapple juice-to-water ratios revealed that these ratios significantly affected the wines' overall acceptability. Ratios of 2:1, 1:1, and 1:2 produced acceptability scores of 6.35, 6.08, and 5.58, respectively (Boondaeng et al. 2021), indicating that the concentration of pineapple juice plays a key role in shaping sensory perceptions and consumer satisfaction. In a related study, wines were produced using a fixed ratio of pineapple must to sugar (1:4) across four different formulations: Recipe A used only natural yeast; Recipe B included granulated sugar and natural yeast; Recipe C incorporated both baker's yeast and granulated sugar along with natural yeast; and Recipe D (control) contained only granulated sugar and baker's yeast. The sensory characteristics of the control wine (Recipe D) closely resembled those of natural palm wine, demonstrating that variations in yeast compositions and sugar levels can significantly influence the wine's flavor profile and preservation qualities. According to Idise (2012), pineapple wines produced using Recipes A through C are suitable for immediate consumption or preservation through refrigeration, highlighting the flexibility of pineapple winemaking methods to suit various taste preferences and storage conditions. This evidence highlights the importance of ingredient ratios and fermentation techniques in pineapple wine production, influencing its sensory qualities, consumer appeal, and storage potential.

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GENERATIVE AI STATEMENT

This manuscript was prepared with the assistance of ChatGPT (OpenAI; GPT-5.2) to improve clarity and language. All scientific content, data interpretation, and conclusions are the author's original work.

ETHICAL CONSIDERATIONS

The study followed both institutional and national ethical guidelines for research involving human participants. Approval from the Institutional Ethics Review Committee (IERC) was obtained before conducting the research. All participants, who were food technologists, were informed about the study's goals, procedures, and potential risks. Informed consent was collected from all participants to ensure voluntary participation and data confidentiality. The study did not include minors or individuals below 18 years of age.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

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