

Study on Bioactive compounds and antioxidant activity of wine produced from *Ananas comosus* and *Manilkara zapota* peels

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ABSTRACT:

This study explores the potential of using pineapple (*Ananas comosus*) and chiku (*Manilkara zapota*) peels and waste for wine production, focusing on the impact of different sugars white sugar, brown sugar, and jaggery on the wines quality. The fruit wastes underwent qualitative phytochemical analysis, revealing various bioactive compounds. Wines were fermented with these sugars and assessed for physicochemical properties. Pineapple wine showed superior results compared to chiku wine in overall physicochemical studies. Quantitative analysis of total phenol content revealed that pineapple wine with jaggery (696.2 mg/g) and chiku wine with jaggery (675.8 mg/g) had the highest phenolic content. The DPPH antioxidant assay revealed significant radical scavenging activity in both wine types, with IC₅₀ values of 42.06 µg/ml for *A. comosus* and 44.84 µg/ml for *M. zapota*. HPLC and sensory evaluations indicated that jaggery-enhanced wines had better phenolic content, antioxidant activity, aroma, taste, and overall quality than those made with sugar or brown sugar. This research underscores the potential of using jaggery in fruit wine making to enhance both chemical composition and sensory experience, offering insights for developing high-quality, health-oriented wines. Future research should explore the large-scale production and health benefits of these wines.

Keywords: Wine production, Antioxidant activity, *Ananas comosus*, *Manilkara zapota*

INTRODUCTION:

Fruits are a vital gift from nature, rich in life-prolonging nutrients and phytochemicals. Fermentation, a key biotechnological process, utilizes beneficial microorganisms to enhance food preservation and shelf life while minimizing reliance on modern preservation techniques [1,2]. Regular fruit consumption improves health and reduces disease risk [3]. Recently, interest in fruit wines has surged due to their health benefits, achieved by incorporating medicinal plants, co-fermenting various fruit juices, and adding ingredients like ginger [4]. Fruit wines contain beneficial phenolic compounds, including flavonoids and organic acids, which offer antioxidant, anticancer, and antimicrobial properties [5].

Wine, a significant non-distilled fermented beverage, is typically made from grapes but can also be produced from other fruits like pomegranate, apple, and mango. With high fruit waste during peak seasons, fermenting excess fruits into wine presents an effective preservation method, extending shelf life compared to jams or juices.

This practice not only reduces post-harvest losses but also diversifies wine options, making it a sustainable solution for managing fruit surplus [6]. The pineapple (*Ananas comosus*) and chiku (*Manilkara zapota*) are both tropical fruits with significant health benefits. Pineapple is rich in beneficial phytochemicals but has a short shelf life, resulting in 15–20% post-harvest losses. Chiku offers essential vitamins and minerals. Both fruits represent promising alternatives for winemaking, emphasizing their therapeutic properties and the necessity of developing postharvest technologies to improve their marketability [7]. This research focuses on the production of wine from pineapple (*Ananas comosus*) and chiku (*Manilkara zapota*) wastes, aiming to enhance their economic value and explore their therapeutic potentials.

MATERIALS AND METHODS:

Sample Collection:

In this study, peels and waste from *Ananas comosus* and *Manilkara zapota* were collected from local juice shops

and households of Bangalore. The samples were gently washed and then homogenized using a mortar and pestle.

Qualitative Phytochemical analysis:

Based on the study by Radha et al. (2021), the samples underwent qualitative examination to assess their phytochemical content [8].

Preparation of Wine:

For wine preparation, 75 g of the semi-ground pineapple and chiku waste, including peels, were mixed with white sugar, brown sugar, and jaggery, respectively. Each mixture was activated with dry yeast (*Saccharomyces cerevisiae*) in lukewarm water. Fermentation occurred anaerobically in airtight containers for 21 days, followed by one week of aging. This process explored the impact of different sugars on wine characteristics while effectively utilizing agricultural by-products.

Physicochemical Parameters:

The physicochemical parameters, including pH, total suspended solids (TSS), Titratable acidity, Volatile acidity, Specific gravity, Alcohol content, and Total sugars, were determined based on Rashmi Mishra, 2016[9].

Determination of pH:

pH was measured directly during fermentation using a digital pH meter.

Determination of TSS:

Total suspended solids (TSS) were identified with a refractometer (Milwaukee MA871 Refractometer).

Determination of Titratable Acidity:

Titratable acidity was assessed using the Association of Analytical Community's method 962.12. [10] A degassed sample was titrated with 0.1 N NaOH to a pale pink endpoint, calculated as:

$$\text{TA (g tartaric acid/L)} \\ = \text{N NaOH} \times (\text{mls NaOH}) \times 75 / \text{mls of sample}$$

Determination of Volatile Acidity:

Volatile acidity was measured during fermentation using the Association of Analytical Community's International (2000) method 962.12 [10]. The sample was degassed by gentle agitation, and the water's pH was adjusted with phenolphthalein. A 0.05 N NaOH solution neutralized the water to a faint pink color. Then, 5 ml of the sample was titrated with 0.05 N NaOH in a 250 ml flask until the pale pink color persisted for 30 seconds, as per Buick and Holdstock, 2003[11].

Determination of Specific Gravity:

Specific gravity was determined using a relative density bottle, which was washed with tap water, dried, and cleaned with ethanol before drying again. The empty weight of the bottle was recorded (M0). Then, 5 ml of the wine sample was added, and its weight was measured (M1). Next, the weight of the empty bottle plus 5 ml of distilled water was recorded (M2).

Specific gravity was calculated as follow:

$$\text{Specific gravity} = \frac{\text{Weight of volume of sample (M1 - M0)}}{\text{Weight of an equal volume of water (M2-M0)}}$$

Determination of Alcohol Content:

Percentage of alcohol was identified with hydrometer Alcohol by volume (ABV).

$$\text{ABV \%} = (\text{Initial Gravity} - \text{Final Gravity}) \times 131.25$$

Determination of Total Sugars:

Total sugars were measured using the Lane and Eynon volumetric method, titrating the sample against boiling Fehling's solution and expressing the results as a percentage. Centrifuged and near-neutral samples were used for estimating reducing sugars, which were also expressed as a percentage (w/v) [12].

Determination of Total phenolic components (TPC):

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method [13]. Sample extracts (20–100 μ l) were mixed with distilled water, followed by 150 μ l of FC reagent. After 5–10 minutes, 500 μ l of 20% sodium carbonate was added and incubated in the dark for 1 hour. Absorbance was measured at 517 nm, with results expressed as mg gallic acid equivalents (GAE)/g of sample.

Determination of Antioxidant activity by DPPH Method:

The antioxidant activity was assessed using the DPPH method [13]. Sample extracts (20–100 μ l) were diluted in phosphate buffer and combined with 0.1 M tris HCl. DPPH solution was added to each test tube, with controls using ethanol and ascorbic acid. Absorbance was measured at 517 nm. Radical scavenging activity was calculated, and the IC50 value was estimated using sigmoid non-linear regression, with all determinations conducted in triplicate [14].

Sensory Evaluation:

Sensory evaluation involved 15 participants assessing the wine's visual appearance, aroma, taste, mouthfeel, and overall harmony, following OIV International

HPLC Analysis:

HPLC analysis was performed using a Waters Spherisorb column and a photodiode array detector to identify phenolic compounds in pineapple waste wine. Compounds were separated on an Acclaim® C18 column at 30°C, using a mobile phase of acetonitrile, acetic acid solution (pH 3.0), and methanol, at a flow rate of 1 ml/min with a 20 µl injection volume. Standard solutions of phenolic compounds were prepared in methanol.

RESULT AND DISCUSSION:

Sample collection and Wine preparation:

For this research, samples of pineapple (*A comosus*) and chiku (*M zapota*) were collected from local juice shops and homes, utilizing the flesh left after juice extraction as well as the peels. The wines were prepared using similar methodologies with modifications based on Joshi et al., 2009[16]. The inclusion of different sugars (sugar, brown sugar, jaggery) and the regular monitoring of fermentation parameters provide valuable insights into the impact of these variables on the quality of the final wines. Figure 1 showed the steps involved in the wine preparation.



Fig 1. Production of Wine from *Ananas comosus* and *Manilkara zapota*

Phytochemical analysis:

A comosus contains high amounts of tannins, anthocyanins, coumarins, flavonoids, phenolic compounds, and quinones. Similarly, *M zapota* is rich in anthocyanins, coumarins, flavonoids, phenolic compounds, and quinones, while other phytochemicals are present in trace amounts. According to Sourabh Pujari et al., 2021[17], pineapple is a good source of various phytochemicals, including flavonoids, coumaric acid, ellagic acid, ferulic acid, chlorogenic acid, as well as micronutrients and dietary fibers.

Physicochemical parameters of *Ananas comosus* and *Manilkara zapota* wines:

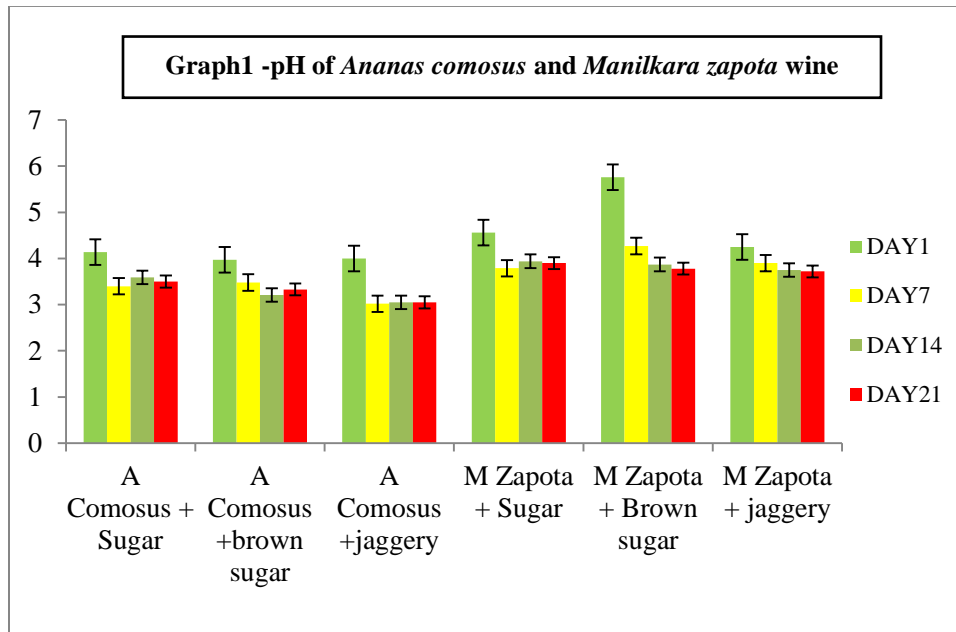
1. pH Levels of the Wines:

The analysis of *A comosus* and *M zapota* wines highlights the impact of fermenting agents on pH levels and fermentation conditions. For the *A comosus*, wine with jaggery (Sample 3) displayed a more acidic pH (3.05) after 21 days, indicating a potentially less favorable environment for yeast compared to samples using sugar (3.5) and brown sugar (3.33) (Graph1).

Similarly, pH of the wine of *M zapota* with jaggery (Sample 3), decreased from 4.25 to 3.72, while samples

with sugar (3.9) and brown sugar (3.78) remained more stable and closer to the optimal range for yeast activity (4.5 to 6.5). This suggests that jaggery-based wines may

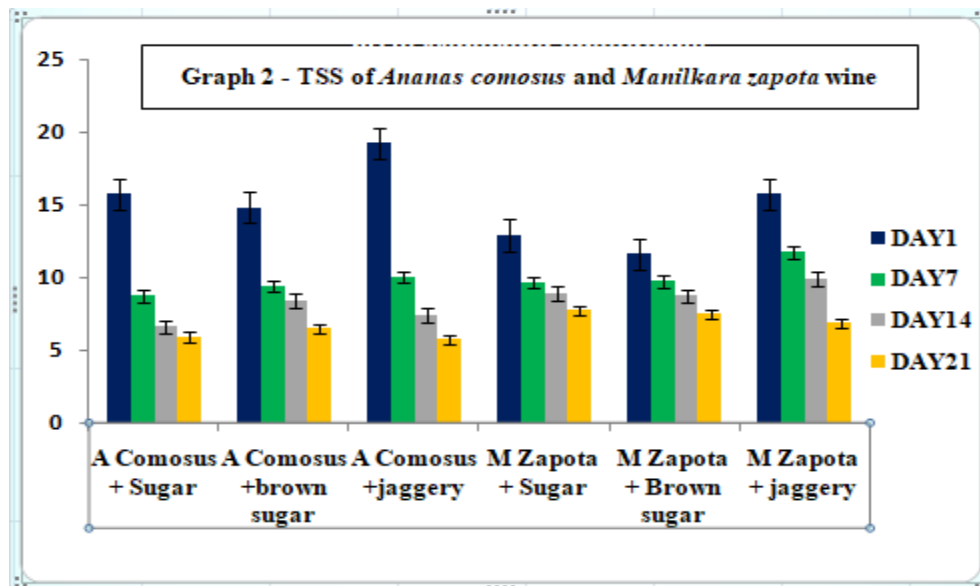
pose fermentation challenges due to lower pH, ultimately affecting yeast growth and ethanol production.



2. Total Suspended Solids (TSS):

The analysis of TSS in *A comosus* and *M zapota* wines indicates effective clarification with Sample 3. In *A comosus*, TSS decreased from 19.20 to 5.7 over 21 days with this sample. It suggests the efficient sedimentation, while sugar and brown sugar wines remained stable at

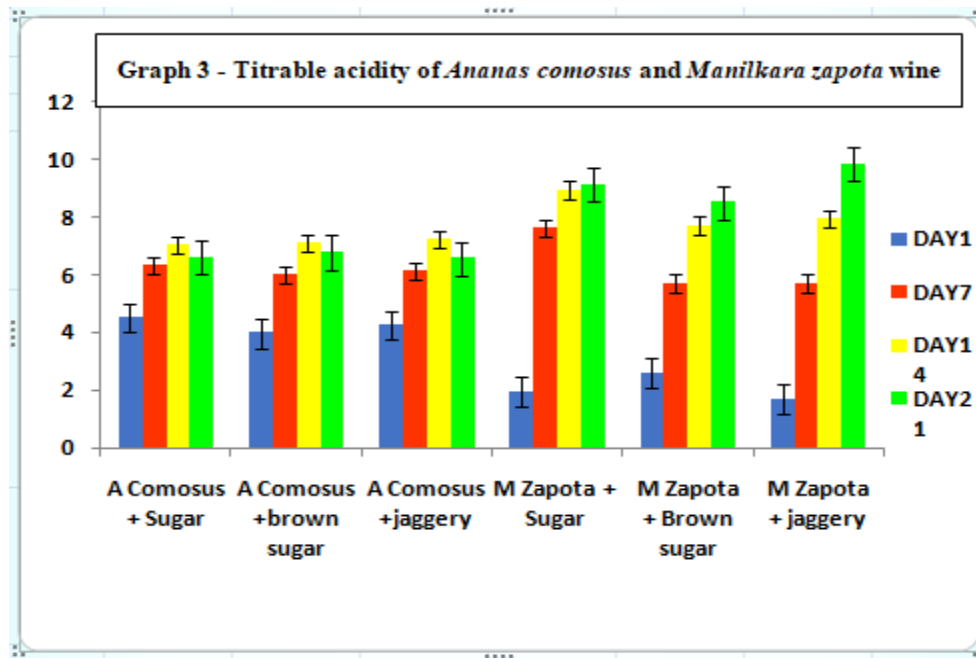
5.9 and 6.5. Similarly, in *M zapota*, TSS for Sample 3 ranged from 15.7 to 6.83, compared to 7.71 and 7.5 for sugar and brown sugar (Graph 2). Higher TSS values could indicate more residual sugars or less effective sedimentation compared to jaggery-based wine.



3. Titratable Acidity (TA):

The analysis of titratable acidity (TA) in *A comosus* and *M zapota* wines highlights significant differences showed in graph 3. For *A comosus*, Sample 3 showed fluctuating TA values (4.25 to 6.56), while sugar

(Sample 1) and brown sugar (Sample 2) maintained consistent values (6.6 and 6.77). In *M zapota*, (Sample 3) ranged from 1.7 to 9.83, contrasting with stable values of 9.1 and 8.5 for sugar and brown sugar. Titratable acidity is crucial for microbial stability and flavor in wine.

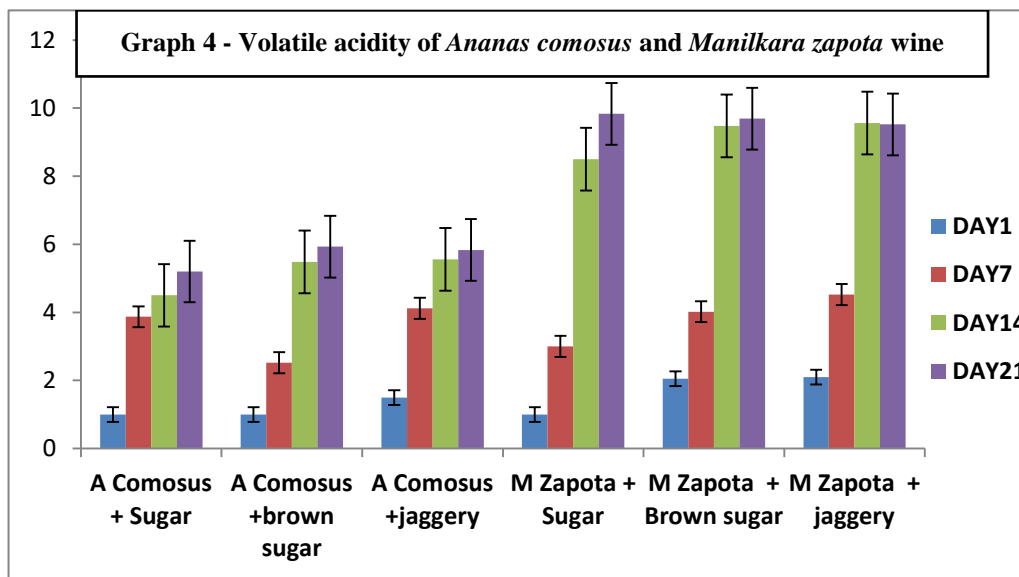


4. Volatile Acidity (VA):

Volatile acidity (VA) is an important quality parameter in winemaking, significantly influencing sensory characteristics. High VA levels can lead to undesirable vinegar-like aromas, affecting the overall quality and consumer acceptance of the wine [11].

There was variation in the VA levels in *Ananas comosus* wine, which ranged from 1.5 to 5.2. On the other hand, wines made with sugar and brown sugar (Samples 1 and 2) showed more consistent VA levels, 5.932 and 5.85,

respectively. Sample 3 appears to have a more favorable sensory profile, as indicated by the lower VA values. The VA of *M zapota* wine with jaggery (Sample 3) value varied from 2.1 to 9.52. Samples 1 and 2 that used sugar and brown sugar produced 9.83 and 9.69 of VA respectively. The increased VA levels linked to brown sugar and sugar point to a tendency toward increased acidity, which may take away from the wine's sensory appeal.



5. Specific Gravity (SG):

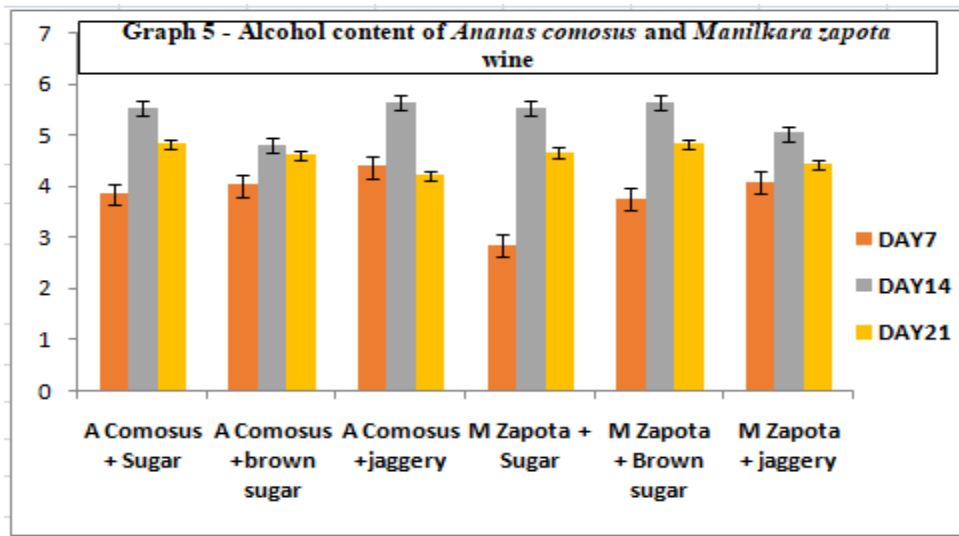
The SG values for *A comosus* wine, (Sample 3) and brown sugar (Sample 2) were similar at 0.98, while the sugar-based wine (Sample 1) had a slightly higher SG of 0.99. Specific gravity indicates the density of the wine and helps estimate alcohol content.

In *M zapotawine*, SG for jaggery (Sample 3) ranged from 1.022 to 0.98, Hence there is no significant difference between *M zapotawine* using sugar (Sample 1) and brown sugar (Sample 2) at 0.99 and 0.98, respectively. A decreasing SG reflects the progression of fermentation and alcohol production.

6. Alcohol Content:

Sample 3 *A comosus* wine had alcohol content ranging from 0% to 4.2%, while those with sugar and brown sugar (Samples 1 and 2) had higher alcohol levels of 4.82% and 4.61%, respectively (Graph 5). Typical wine alcohol content ranges from 8% to 15% ABV (alcohol by volume) [9].

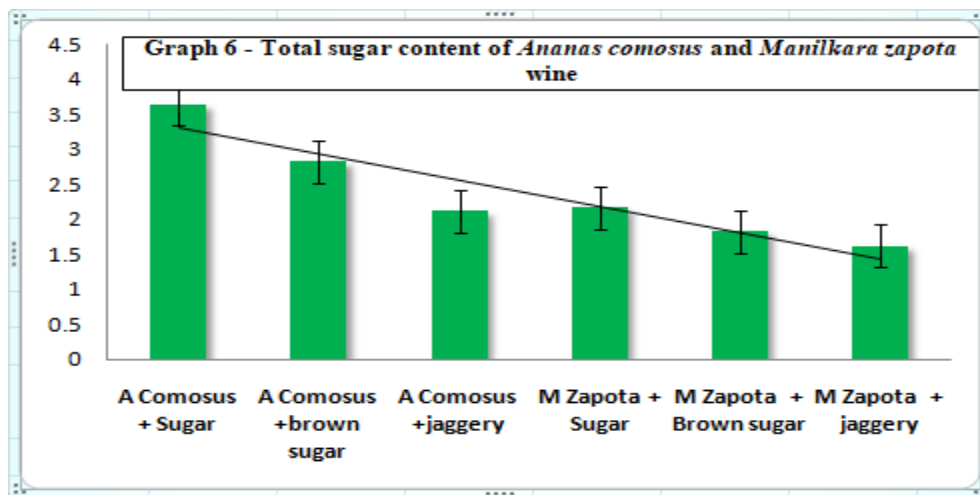
For *M zapotawine*, alcohol content for jaggery (Sample 3) varied from 0% to 4.43%, whereas sugar (Sample 1) and brown sugar (Sample 2) produced 4.66% and 4.82% alcohol, respectively. The observed alcohol levels are relatively low compared to many other wines [(L. V. A. Reddy et al., 2014)].



7. Total Sugar Content:

In *A comosus* wine, total sugar levels for jaggery (Sample 3) were 2.12, compared to 3.65 for sugar and 2.83 for brown sugar-based wines. Sugar content influences fermentation and sweetness (S. S. Nielsen,

2010). In *M zapota* wine, total sugars were 1.62% for jaggery, 2.17% for sugar, and 1.83% for brown sugar (Graph 6) . These values reflect the residual sugars affecting the final sweetness and mouthfeel of the wine.



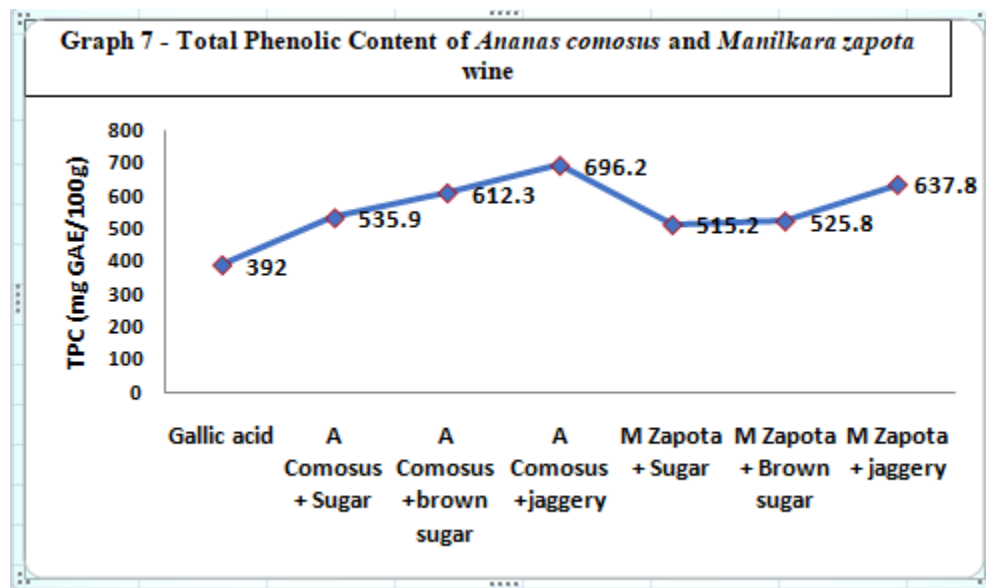
The analysis reveals that different fermenting agents have varying impacts on the physicochemical parameters of both *Ananas comosus* and *Manilkara zapota* wines. Jaggery tends to create more acidic conditions and higher phenolic content, potentially influencing taste and aging properties. Sugar and brown sugar generally provide more stable fermentation conditions, leading to more consistent pH, TSS, TA, and alcohol content.

Determination of Total Phenolic Contents (TPC):

The total phenolic content of *A comosus* wine, measured using the Folin–Ciocalteu method, was highest in wine

made with jaggery (696.2 mg/g), followed by brown sugar (612.3 mg/g), sugar (535.9 mg/g), and the standard gallic acid (392.0 mg/g). This indicates that jaggery significantly enhances the phenolic content compared to other sugars used.

Similarly, the total phenolic content in *M zapota* wine, determined by the same method, was also highest in the wine made with jaggery (637.8 mg/g). This was followed by brown sugar (525.8 mg/g), sugar (515.2 mg/g). These phenolic contents of both wines samples were significantly differ from standard gallic acid (392.0 mg/g) level (Graph 7).



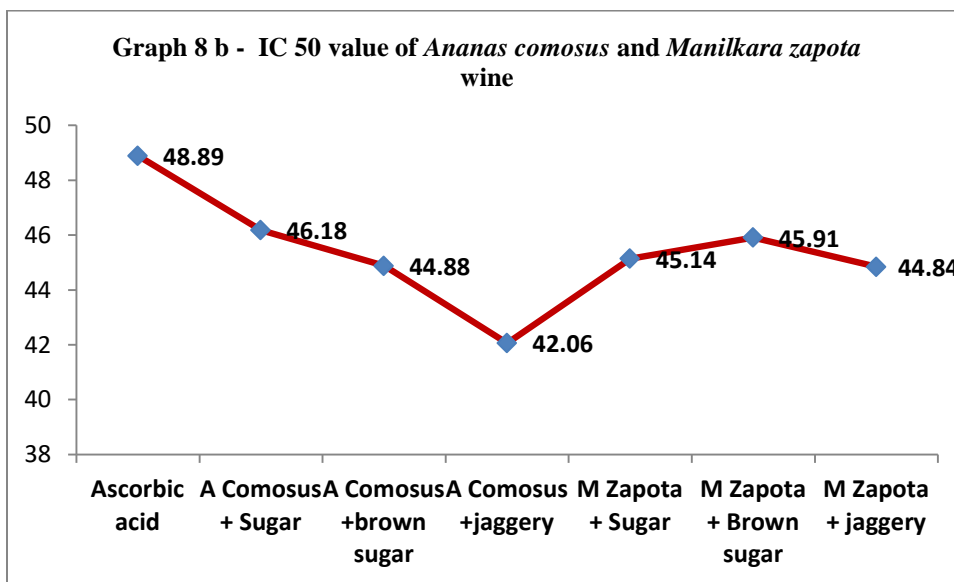
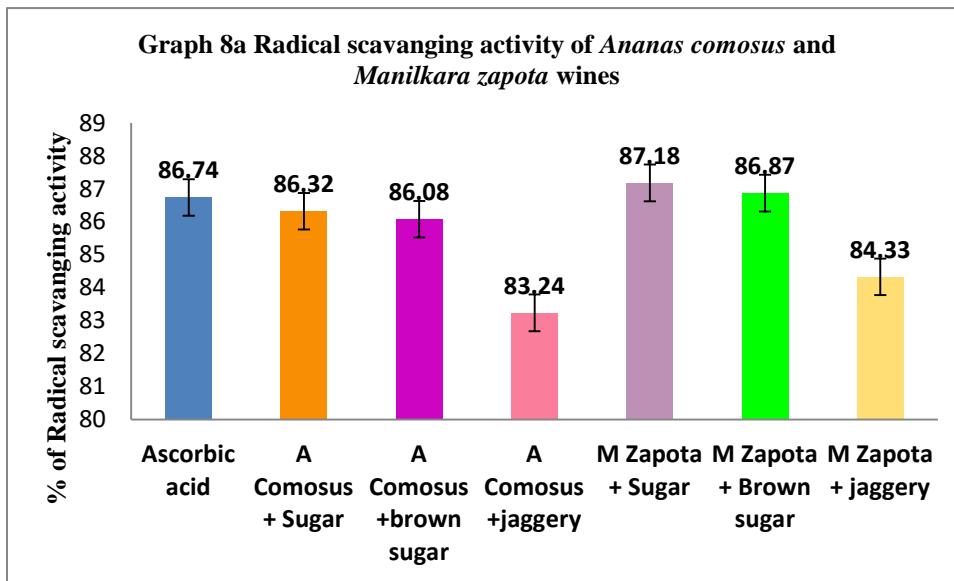
These results demonstrating that jaggery consistently leads to the highest phenolic content across both fruit types. This suggests that jaggery not only enhances the phenolic compounds in both types of wine but also contributes to their potential health benefits and stability [18,19].

Free Radical Scavenging Activity – DPPH method:

The In-vitro antioxidant assay was performed using the DPPH method. The results indicated that wine samples from *A comosus* and *M zapota* (Sample 3) exhibited significant antioxidant activity (83.24 µg/ml and 84.33 µg/ml, respectively) compared to the standard ascorbic acid at the highest concentration (100 µg/ml), which showed an activity of 86.74 µg/ml, as illustrated in Figure 8a. All samples from both wine groups

demonstrated radical scavenging activity, although no significant difference was observed within the groups.

The IC50 value derived from the calibration curve of standard ascorbic acid ($y = 0.388x + 16.54$) was found to be 48.03 µg/ml. This value significantly differs from the IC50 values of *A comosus* and *M zapota* wines prepared with jaggery, which were 42.06 µg/ml and 44.84 µg/ml, respectively. There was no significant difference observed between *Ananas comosus* wines prepared using sugar and brown sugar (46.18 µg/ml and 44.88 µg/ml, respectively). Similarly, *Manilkara zapota* wines made with different sugars showed no significant difference in IC50 values (45.14 µg/ml and 45.91 µg/ml), as depicted in Graph 8b.



Both *A comosus* and *M zapota* wines exhibit significant antioxidant activity, with jaggery-based wines showing superior free radical scavenging. This enhanced activity is linked to higher phenolic content from jaggery, which is more effective than sugar or brown sugar in boosting antioxidant levels. Francesca Melini and Valentina Melini, 2021 highlighted that fermentation increases phenolic compounds and antioxidant capacity [19].

High Performance Liquid Chromatography (HPLC) Analysis:

Based on biochemical research, it is believed that jaggery-based *A comosus* wine has a positive effect.

Thus, only these samples were subjected to HPLC analysis.

The HPLC analysis revealed that the standard gallic acid peak appeared with a retention time of 6.18 minutes and an area of 3,877,021 (Fig 2a). In contrast, *A comosus* wine (sample C) showed a peak at 6.13 minutes with an area of 76,048 (Fig 2b). Vívian Maria Burin et al., (2021) validated an HPLC method for red wine polyphenols, noting a narrower linear range for p-coumaric and ferulic acids (0.3-30 µg/mL) compared to this study's range [20, 21].

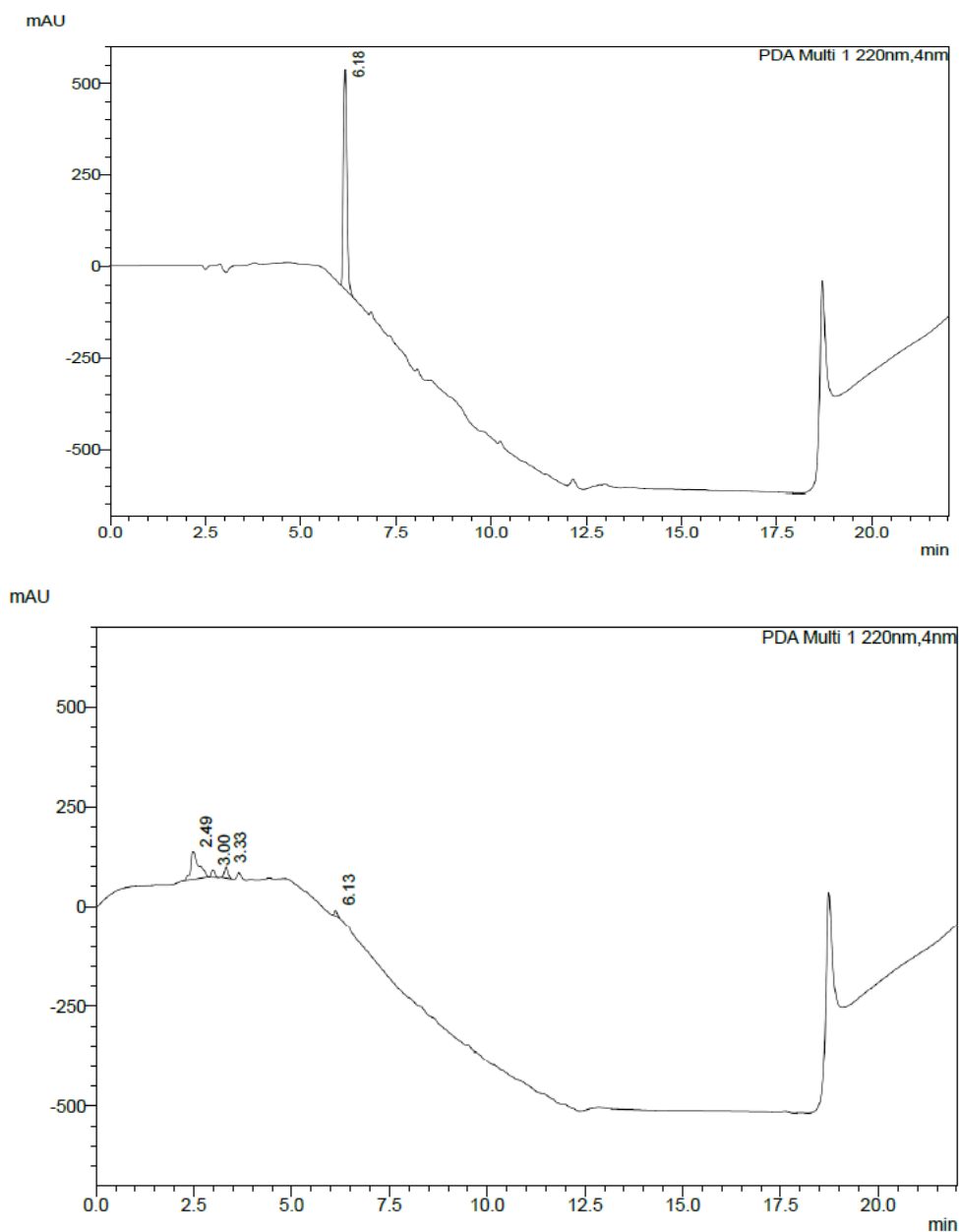


Figure 2 HPLC chromatogram of a. Standard Galic acid b. *A comosus* jaggery wine

Sensory Evaluation:

The sensory evaluation of the wine is shown in the table 1. *A comosus* wine demonstrated better sensory scores compared to *M zapota* wine. In both cases, the use of jaggery consistently resulted in the highest sensory evaluations. Jaggery-based wines outperformed

those made with sugar and brown sugar in visual appeal, aroma, mouthfeel, and overall harmony. This suggests that jaggery not only enhances phenolic content but also significantly improves the sensory attributes of both wines, indicating their potential for high-quality production and consumer preference.

Wine Variety	Visual (2.0)	Aroma(2.0)	Taste(2.0)	Mouth feel (2.0)	Harmony—overall appraisal(2.0)	Final score (10)
<i>A Comosus</i> + Sugar	1.5	1.6	1.7	1.5	1.8	8.1
<i>A Comosus</i> +brown sugar	1.7	1.7	1.9	1.7	1.8	8.8
<i>A Comosus</i> +jaggery	1.8	1.9	1.9	1.7	1.9	9.4
<i>M Zapota</i> + Sugar	1.5	1.5	1.5	1.7	1.7	7.9
<i>M Zapota</i> + Brown sugar	1.6	1.7	1.8	1.8	1.8	8.1
<i>M Zapota</i> + jaggery	1.7	1.9	1.7	1.8	1.9	9

Table 1 - The sensory evaluation of the *Ananas comosus* and *Manilkara zapota* wine

CONCLUSION:

The comparative analysis of pineapple (*Ananas comosus*) and chiku (*Manilkara zapota*) wines revealed that the type of sugar used during fermentation significantly influences various physicochemical parameters and overall wine quality. Jaggery-based wines exhibited lower pH levels, higher total phenolic content, and enhanced antioxidant activity compared to those made with sugar and brown sugar. Specifically, *A comosus* wine with jaggery demonstrated the highest total phenolic content (696.2 mg/g) and antioxidant capacity (IC₅₀ value of 42.06 µg/ml), suggesting substantial health benefits.

Moreover, while jaggery created more acidic conditions that could hinder yeast activity, it ultimately enriched the sensory profile, resulting in better aroma, mouthfeel, and overall harmony in sensory evaluations. The wines produced with sugar and brown sugar maintained more stable physicochemical properties, indicating their suitability for consistent fermentation and higher alcohol content.

The results underscore the potential of using jaggery not only for enhancing the health benefits and stability of fruit wines but also for improving sensory attributes, which could appeal to consumers. Overall, this study highlights the importance of sugar type in winemaking and suggests further exploration into optimizing fermentation conditions to harness the benefits of jaggery for high-quality wine production.

Future research should explore the biochemical studies and health benefits of these wines. This will aid in refining industrial production methods and exploring cost-effective fermentation techniques for large-scale manufacturing.

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Conflicts of interest:

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