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Standardizing protocols for fermented beverages from papaya (*Carica papaya* L.)

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Abstract
This study investigates the development of papaya wine as a novel fruit-based fermentation product and explores the various steps involved in the wine making process. The objective was to evaluate the various parameters like sugar content, pH and microbial cultures for the development of a palatable papaya wine, with a focus on its sensory attributes, nutritional, and overall quality. Different levels of sugar (15, 30 and 45 percentage), pH (3.0, 3.5, and 4.0) and different microorganisms (*Saccharomyces cerevisiae*, *Lactobacillus plantarum*, *Leuconostoc oenos*, *Pediococcus cerevisiae*, *Acetobacter xylinum and Williopsis saturnus)* were tried for standardizing the protocols to achieve early and better quality wine. The sensory analysis performed to evaluate the consumer preference revealed that wine developed with the help of *Saccharomyces cerevisiae* and *Williopsis saturnus* at 30 % sugar and pH 3.5 had maximum consumer acceptance. The physiochemical parameters such as TSS, pH, Titrable acidity, Total sugar, reducing sugar, alcohol %, specific gravity and total phenolic content of the wine samples were analysed. Proximate analysis of the samples for Vitamin A, Iron, Protein, carbohydrate and fat was also determined. Among the various treatments, those with 45 % sugar, pH-4.0 and the organism *Williopsis saturnus* has yielded a wine product with the highest alcohol content and nutritional quality.

Keywords: papaya, wine, fermented beverages, proximate analysis

1. Introduction
Papaya (*Carica papaya*) is a tropical fruit renowned for its unique flavour, nutritional value, and versatile culinary applications. Papaya fruits have good market demand due to easy availability, lower price, high palatability, and nutritional and medicinal benefits (Azad *et al*., 2012) [6]. Papaya fruits have a low sugar content and high acid content (Cheng *et al.*, 2016) [10]. The fruits are an excellent source of beta carotene (2020 IU/100 g) (Aravind *et al*., 2013) [4] and essential nutrients such as Iron, Calcium, Vitamin B and C. Papaya fruits enhance digestion, promote wound healing and are also beneficial against diabetes, cancer, heart attack and blood pressure.

While papaya is commonly consumed fresh or in various processed forms, such as jam, pickle, tutee fruity, candy etc., its potential for winemaking remains relatively unexplored compared to its easy availability and low market price. When papaya is grown in homesteads, most of them get ripen and rot, many fruits go unutilised or underutilized. Value addition of Papaya fruits which could not be consumed fresh, to fermented beverages provides an opportunity to minimize post-harvest losses. This will also enhance income and nutritional security.

Papaya possesses several qualities that make it an attractive choice for winemaking. Firstly, papaya exhibits a complex flavour profile, combining sweetness with nutritional qualities. Papaya is a rich source of essential nutrients, and antioxidants, which may contribute to the potential health benefits associated with moderate wine consumption. They also have a moderate to high content of sugars depending on the variety. These characteristics lend papaya emerging as a promising candidate for the development of fruit-based wines.

Fermentation is a comparatively low-energy food preservation method that lengthens shelf life and eliminates the need for refrigeration or other food preservation methods. Wine is a fermented beverage, which plays a vital role in human life, providing social, religious, and economic advantages. Demand for fruit based low-alcoholic wines are increasing because they are healthier and have distinctive flavour, aroma, and colour that appeal to the global wine market, winemakers and consumers (Samec *et al*., 2016) [32].

The development of papaya wine requires a systematic approach, and providing proper environment for fermentation is crucial to ensure the sweetness, quality and consistency of the final wine product.

A combination of several factors, including optimum sugar content and acidity is essential to create an ideal environment for yeast fermentation and the relative sweetness of the wine.

Sugar content is a crucial factor in fruit wine fermentation as it serves as the primary substrate for yeast fermentation leading to alcohol production. Studies have indicated that an optimal sugar content within a specific range promotes yeast activity and ensures the desired sensory profile of the wine (Zombardo, *et al.*, 2020) [39]. Bryan *et al*., (2018) [8] reported that varying sugar concentrations in fruit juices significantly influence the kinetics of fermentation, yeast viability, and production of volatile compounds, ultimately influencing the sensory attributes of the wine.

The fermentation of original jackfruit juice of 14 % w/w sugar concentration using 0.5% w/v yeast for 9 days was the best to produce a good quality wine with 12.13% v/v of ethanol and specific jackfruit aroma (Kumoro *et al*., 2012) [21]. In a study with different levels of sugar such as 200g, 250g, 300g, 350g, 400g, 450g, 500g, and 550g for developing Grapefruit wine, those with 300g sugar has performed best for TSS, acidity, alcohol content, specific gravity, and sensory attributes like colour, appearance, taste, aroma and overall acceptability when compared to all other treatments (Mishra *et al*., 2022) [27]. In another study, 5 different levels of sugar *viz*., 20°, 25°, 30°, 35°, 40° and, 45° brix were tried for formulating pineapple wine along with 700 ml pineapple juice, 35g of beetroot extract and 0.122% wine yeast (Kumar *et al*., 2022). Among these treatments, pineapple wine with 35° brix has performed best in nutritional parameters such as TSS, acidity, pH, alcohol content, specific gravity, colour and sensory attribute such as appearance, taste, aroma and overall acceptability when compared to all other treatments. Among the three levels of sugar *viz*., 22, 24 and 26 °Brix added with pulp for developing Mulberry wine, those prepared with 26 °Brix sugar has recorded the highest sensory score and regarded as the best wine (Ghan *et al*., 2015) [17]. However, Umeh *et al*., (2015) [36] reported that papaya wine can be successfully produced at low sugar levels.

In wine production, the pH of the fruit pulp/juice has an impact on fermentation rate, stability, and flavour, fragrance, and colour. Low pH prevents the development of undesirable microorganisms and can therefore enhance the quality of the final product (Satav and Pethe, 2016). The acidic pH encourages a greater production of alcohols. Lack of acidity is bound to slow down fermentation and result in an inferior product. In a study for development of papaya wine using *Lactobacillus plantarum*, and *Pediococus pentosaceus*, Awe (2011) observed that the pH range increased from 3.2 to 3.6 as a result of yeast metabolism. In a study on development of papaya wine by Cholassery, *et al*., (2019) [11], it was observed that alcohol level increased as the pH decreased with progress in fermentation. Alcohol content was 3.01% at a pH range of 4.95 on the fifth day, while alcohol content on the twentieth day had risen to 10.11% at a pH range of 4.45.

Wine fermentation may be either natural with innate wild yeasts, or artificial using yeast cultures such as Baker's yeast (Emmanuel and Odoyo, 2011) [15]. *Sacchromyces cerevisiae* strains are used most frequently in the production of fruit wines because they enable quick and consistent fermentation while lowering the risk of slow or blocked fermentation and microbial contamination (Duarte *et al.*, 2010) [13]. Due to its high degree of metabolic activity and tolerance of high alcohol concentrations, *Saccharomyces* is the organism preferred for fermentation of wine products (Ezemba, and Archibong, 2017). Papaya juice was successfully fermented by *Saccharomyces cerevisiae* to produce wine. The most important factors affecting ethanol production were total soluble solids (TSS), process temperature, pectinase, inoculum level, and pH (Alagesan and Panneerselvam, 2016). The ideal process of fermentation for wine using Saccharomyces cerevisiae were 24 º Brix TSS, 26 º C temperature, 5 ml of pectinase enzyme, 10% inoculum, and pH 4.5. The ethanol production, which was experimentally verified to be between 11 and 12%, was found to be comparable to these ideal conditions. (Alagesan and Panneerselvam, 2016) [3].

Williopsis saturnus generate large number of esters and produce desired volatiles and it will improve the fruity flavour and provide special oenological traits of wines (Lee *et al*., 2012). *Lactobacillus* and *Pediococcus* strains are typically more tolerant to high ethanol concentrations (Davis *et al*., 1988). *Lactobacillus plantarum* CICC21805 is a functional probiotic with great fermentation ability and abundant metabolites (Wang *et al*., 2021). Recent studies have confirmed that *Pediococcus* species can flourish in wines that are thought to be microbiologically stable. Additionally, *Pediococcus spp*. in wines does not cause deterioration (Wade *et al.*, 2018).

The present investigation involves comparisons between different sugar and pH levels and the efficiency of microorganisms to assess the potential variations in wine production and quality and fixing the protocols through sensory evaluations, proximate and physicochemical analysis. Keeping this in view, the present investigation was proposed for the optimization of process parameters for the development of papaya wine.

2. Material and Methods

An experiment was conducted at the School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore, Tamil Nadu, India for Standardizing protocols for fermented beverages from Papaya (*Carica papaya* L.) as part of the PG programme during 2022 -2023.

Papaya variety 'Red lady' which is pink fleshed and contains 63.5% lycopene, is selected for the study. Mature ripe red lady papaya fruits were procured from local market in Coimbatore. The fruits were properly cleaned under running water and stored in the post - harvest laboratory until it reached the correct stage of ripening.

A basic recipe was followed for the formulation of wine with the ingredients (1). Fruit pulp 500g (2) Sugar source 250g (50%) (3) yeast/microorganism 2g and total volume made up to 1 litre.

Sugar source such as honey was procured from local bee keepers and palm jaggery was procured from Coimbatore local market.

2.1. Preparation of Wine

The papaya fruits were rinsed thoroughly with running water. The skin was peeled out, the flesh chopped into small pieces and blended in the form of a pulp using a blender. The sugar source honey (125 ml) and jaggery (125 g) was dissolved in equal quantity of water (250 ml) after boiling for 5 minutes and allowed to cool. About 2g of yeast/ microorganism was mixed with 25 ml warm water and kept for 15 mins, till

bubbling is complete. This yeast/ microbial culture is added to the Fruit pulp and mixed well. Sugar solution is added to this mixture and stored in a glass jar. The mixture is stirred every day until 14 days and then kept undisturbed for another one week. After 3 weeks, when the fermentation process is complete and the aroma and flavour profiles develop, the wine sample is filtered with a soft mesh cloth removing the froth and sediments. It was then stored in a glass bottle for ageing of wine. The standard procedure for papaya wine is mentioned in fig.1

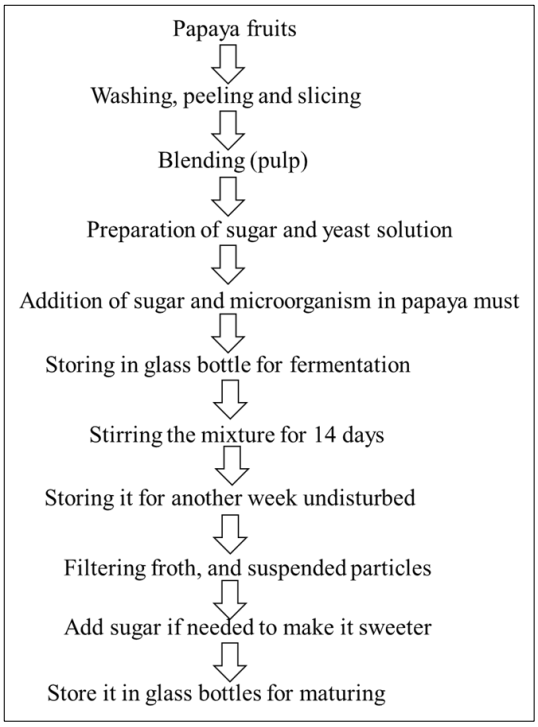

Fig 1: Flow chart for papaya wine production

Experiment 1: Effect of pH and sugar levels for papaya wine production

In this experiment, a combination of different level of pH (3.0, 3.5 and 4.0) and sugar content (15, 30 and 45 %) was tried. The pH values of fruit must was adjusted using digital pH meter with standard buffers (pH 4 and 7). In this experiment Baker's yeast was used for fermentation.

Experiment 2: Efficiency of different microbial cultures for papaya wine production

In this experiment, different microbial cultures were employed for fermentation and preparation of wine. The organism such as Saccharomyces cerevisiae (NCIM Acc No 3662), Lactobacillus plantarum, (NCIM Acc No 2374), Leuconostoc oenos (NCIM Acc No 2219), Acetobacter xylinum (NCIM Acc No 2526), Williopsis saturnus (NCIM Acc No 3163) were procured in the form of active slant from NCIM, CSIR-NCL, Pune. Pediococcus cerivisiae (NCDC No 38) was procured from NCDC, NDRI, Karnal. By using Nutrient Agar media these cultures are made into broth culture by using wire loop. 1 loop of broth culture of organisms is used for fermentation for each sample.

2.2 Analysis of the product

The samples were subjected to Sensory analysis as well as Physiochemical, Proximate and Microbiological analysis

2.2.1 Sensory analysis

The papaya wine samples developed were subjected to sensory evaluation. A semi-trained panel of 15 judges were selected at random consisting a heterogenous group of students, faculty and public for evaluating the product for appearance, colour, taste, aroma, texture, flavour, bitterness, strength and overall acceptability on a 9-point hedonic scale (Lawless *et al*., 1997). Panellists fall on the age group of 18-60 years. The panellists were asked to rate the samples for a number of attributes on a Likert scale from 1 to 9, where 1 was the least preferred and 9 the most preferred of the attribute characteristics. (Meilgaard *et al*., 2006) [25]

2.2.2 Physiochemical Analysis

2.2.2.1 Total Soluble Solids (TSS)

A hand Refractometer was used for measuring Total Soluble Solids. The prism was well dried by using a blotter paper. Few drops of wine were applied in the lens and the readings in the degree brix were recorded.

2.2.2.2 Determination of pH:

The pH of the samples was determined by digital pH metre with standard buffers (pH 4 and 7). 10 ml of wine sample was transferred to sterile beaker, and the pH of the wine was determined digitally. (Ranganna, 1986) [30].

2.2.2.3. Determination of Titratable Acidity

The titratable acidity was determined by Titration method. 5ml of the wine sample was pipetted into a 250 ml conical flask, 100 ml of distilled water was added to the flask. 0.1 N NaOH was titrated against the content of the flask until the pale pink colour endpoint is achieved. Titratable acidity was calculated as follows:

$$\% \text{ Tartaric acid} = \frac{E.W\ of\ acid \times \text{Titer} \times \text{Normality of NaOH} \times \text{Volume made up} \times 100}{1000 \times \text{Aliquot taken} \times \text{Weight of sample}}$$

2.2.2.4 Determination of Total sugar

The total sugars were determined by the Anthrone reagent method using colorimetry. Samples were treated with HCl, Conc.H_2SO_4 and anthrone reagent to form blue green coloured compound the absorbance of which was read at 630 nm wavelength and recorded (Hedge and Hofreiter, 1962).

$$\text{Carbohydrate (\%)} = \frac{sugar\ value\ from\ graph\ (mg)}{Aliquot\ sample\ used\ (0.5\ or\ 1ml)} \times \frac{Total\ vol.of.extract}{Wt.of.sample\ (mg)} \times 100$$

2.2.2.5 Determination of reducing sugar

The total sugars were determined by using DNSA method (Dinitro-salicylic acid reagent). Samples were treated with DNSA, crystalline phenol sodium sulphite and sodium potassium tartarate and measure the absorbance at 510 nm wavelength (Miller, 1972).

$$100 \text{ ml of sample} = \text{Absorbance value} \times \frac{100}{1.5} \mu g\ of\ reducing\ sugars$$

2.2.2.6 Determination of Alcohol %

The percentage of alcohol were determined by using Potassium dichromate method using chromic acid. The samples were treated with conc.sulphuric acid and measure the absorbance at 600 nm wavelength. The alcohol % was observed directly from the spectrophotometer. (Cappuccino,

1999) [9] from the standard curve.

2.2.2.7. Determination of Specific Gravity

The specific gravity of wine was determined by using Hydrometer at room temperature. Samples were taken in a measuring cylinder and the hydrometer is spinned inside the measuring cylinder which floats off the bottom. Record the value which seen in hydrometer. (Son, *et al.,* 2009*)* The unit of specific gravity is expressed as g/cm^2.

2.2.2.8. Determination of Total phenolic content

Total phenolic content was determined by Folin's Ciocalteu Reagent Method. Samples were treated with ethanol, FCR, Sodium carbonate and catechol. In an alkaline solution, phenol reacts with phosphomolybdate, an oxidising agent, to produce molybdenum blue, a complex with the colour blue that can be calorimetrically detected at 550 nm wavelength. The Total phenol unit can be derived from standard curve and is expressed as mg/100g (Bray and Thorpe., 1954).

2.2. 3 Proximate Analysis

2.2.3.1 Determination of Vitamin A

Vitamin A was determined by using acetone. The sample was treated with 80% acetone and centrifuged at 3000 rpm. Calculating the beta-carotene concentration using the absorbances at 479, 645, and 663 nm in UV-spectrophotometer and the vitamin A of wine was calculated as follows, (Nagata, 2009).

$$\text{Beta-Carotene (mg/100g)} = 0.854\,\text{Abs.}(479) - 0.312\,\text{Abs.}(645) + 0.039\text{Abs}(663) - 0.005$$

2.2.3.2 Determination of Iron

The iron content of the samples was evaluated using the method described by Raghuramulu, *et al.,* (1983). The samples (20 ml) were burnt to ashes for 2 hours at 550–600 ^{0} C in a muffle furnace. Having been broken down by an acidic solution, after that, potassium persulfate and potassium thiocyanate were applied to the ash sample solutions. According to the sample's iron content, the solution gave off a reddish tint. The O.D. was measured using a spectrophotometer set to 440 nm. A standard curve was made using ferrous ammonium sulphate as the standard. The sample's iron content was calculated as mg of iron per 100 g. The iron content was calculated using the formula below:

$$iron\ mg/100g = \frac{\text{OD of sample}}{\text{OD of standard}} \times 100$$

2.2.3.3 Determination of Protein

Protein was determined by Lowry's method by using Folin-Ciocalteu reagent (FCR) to give blue colour formation when reacted with alkaline copper solution and the absorbance read at 660 nm by spectrometer or Colorimeter (Lowry *et al*., 1951).

Protein content mg of 100 ml of sample = $\frac{absorbance}{0.2\ (or)\ 0.4} \times 100$

2.2.3.4 Determination of Total Carbohydrate

Carbohydrate was determined by phenol-sulphuric acid method. Phenol reagent and sulphuric acid reagent combined with glucose to give green colored product at absorption of 490 nm (Dubois *et al*., 1956) [14].

Absorbance corresponding to 0.1 ml of the test sample =X mg of glucose.

10 ml contains $= X \times \frac{10\ mg\ of\ glucose}{0.1}$ = % of total carbohydrate present.

2.2.3.5 Determination of Total Fat

Total fat was estimated by the Soxhlet apparatus method by Sadasivam and Manickam (1992). A 5 ml of sample is taken and ethanol is used as solvent at 40-60℃ for 16 hrs. Dry the excess of ethanol at 105℃ for 30 mins and weigh the particles left behind.

Crude fat in sample (%) = $(b - 1) \times \frac{100}{wt.of.sample}$

Where, b=final weight of sample.

2.2.3.6. Determination of Calorific value

It was calculated by addition of fat, protein and carbohydrates.

Calorific value = (F×9) + (P×4) + (C×4).

Fat=9 Kcal. Protein= 4 Kcal. Carbohydrates=4 Kcal.

2.2.4 Microbial analysis

Microbial analysis was carried out using standard plate count method, by pour plate technique. According to WHO, the bacterial growth limit and fungal growth limit are 10 CFU/g and 10 CFU/g respectively from 5 days of analysis.

2.2.4.1 Determination of Total Heterotrophic count

Nutrient Agar (NA) was used for the detection of Total Heterotrophic count. A well homogenized wine samples are serially diluted ((10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}).

2.2.4.2 Determination of Yeast and Mould enumeration

Potato Dextrose agar was used for enumeration of yeast and mould. Aliquots (0.1ml from each dilution were taken from well homogenized wine samples that had been serially diluted and transferred to plates aseptically.

2.2.4.3 Determination of Total Coliform count

EMB Agar was used for the detection of coliform count. A well homogenized wine samples are serially diluted ((10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}).

For all the samples, the medium was poured, gently swirled to the left and right, and then left to set. The plates were then kept for 72 hours for incubation. (Adedeji and Oluwalana, 2013) [1].

2.3. Statistical analysis

Factorial Completely Randomized design (FCRD) was adopted for analyzing Experiment 1 and completely randomised design (CRD) for experiment 2. Statistical significance was examined by analysis of variance (ANOVA) and LSDT was performed to compare the means if there is significant difference ($p<0.05$). All analyses were performed using the R Statistical software.

3. Results and discussion

Papaya wine production involves the fermentation of papaya

fruit must to convert the sugars into alcohol. Various factors, such as sugar levels, pH, and microbial cultures significantly impact the process and the quality of the final product.

3.1. Sensory analysis

Papaya wine could be developed as per the standard recipe and a combination of different levels of sugar source involving honey and Jaggery as well as with different pH. The resultant product was subjected to organoleptic evaluation. A semi trained panel of judges consisting 15 members were provided with the vine samples for evaluation of organoleptic characteristics and ranking the products. The average of sensory analysis score recorded by the judges is presented in (Table 1) and in Fig 2.

Table 1: Effect of pH and sugar on the mean sensory score for papaya wine

Sl. No	Parameter	pH 3 sugar 15%	pH3.0 sugar30%	pH3.0 sugar45%	pH3.5 sugar15%	pH3.5 sugar-30%	pH3.5 sugar-45%	pH4.0 sugar-15%	pH4.0 sugar-30%	pH4.0 sugar-45%
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
1	Appearance	6.11	6.33	6.33	6.11	6.33	6.22	6.32	6.95	6.32
2	Colour	6.11	6.22	6.67	6.11	6.33	6.22	6.32	6.66	6.35
3	Taste	5.67	6.22	6.00	5.67	6.11	6.11	6.21	6.74	5.89
4	Aroma	6.56	6.56	6.67	6.44	6.67	6.33	6.37	7.16	6.58
5	Texture	6.56	6.56	6.56	6.11	6.33	6.44	6.43	7.14	6.57
6	Flavour	6.56	6.11	6.22	6.44	6.22	6.46	6.54	6.74	6.36
7	Bitterness	4.00	3.89	4.11	4.00	4.11	3.89	4.46	4.84	4.22
8	Strength	4.89	5.56	4.78	4.56	4.89	6.00	4.87	5.73	4.56
9	Overall Appreciation	6.44	6.33	6.56	6.78	6.11	6.75	6.24	7.17	6.56
	Mean	5.88	5.98	5.99	5.80	5.9	6.05	5.97	6.57	5.93

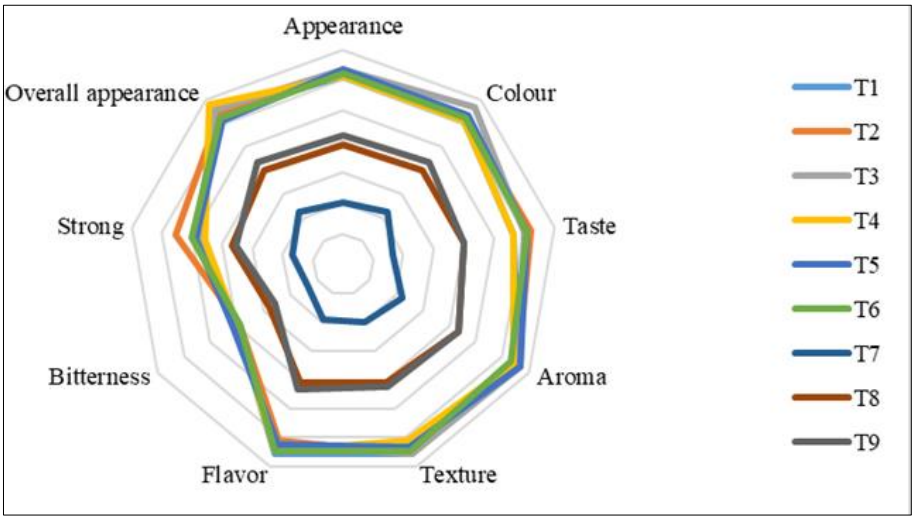

Fig 2: Effect of pH and sugar on the mean sensory score for papaya wine

The highest mean sensory score was received for the wine developed with 30% sugar and pH 4.0. The panellists awarded highest score for most of the parameters included in sensory evaluation like appearance, taste, aroma, texture, flavour, and overall appreciation for this combination. This indicated that when baker's yeast (*Saccharomyces cerevisiae)* is the organism selected for fermentation of papaya, an added sugar percentage of 30% and a pH of 4.0 may work as an ideal condition for the production of a palatable wine.

Sugar concentration plays a crucial role in determining the alcohol content and fermentation efficiency as it serves as the primary energy source for yeast. Higher sugar concentrations provide more fermentable sugars, resulting in increased alcohol production. However, excessively high sugar levels can inhibit yeast activity and lead to osmotic stress and sluggish fermentation. It is important to identify the ideal sugar level to have a right balance for papaya wine production and this objective is met through this experiment.

Similarly, the pH of the papaya pulp influences the activity of the microorganisms affecting fermentation rate and overall stability of the wine. Yeast strains have specific pH preferences for optimal fermentation performance. High pH levels can lead to microbial spoilage, off-flavours, and reduced stability, while low pH levels can inhibit yeast growth and fermentation. Since different fruits/varieties have varying levels of acidity, monitoring and adjusting of the pH during fermentation are essential to maintain optimal conditions for yeast growth and alcohol production.

Yeast, *Saccharomyces cerevisiae*, typically thrives in a pH range of 3.0 to 5.0 (Alabere *et al*., 2020) [2]. In the present study also a pH of 4.0 is found to be ideal. Adjusting the pH within this range can promote yeast growth and fermentation efficiency.

The wine samples developed employing different microbial cultures in Experiment 2 were also subjected to sensory analysis and the mean scores are presented in table 2 and Fig. 3. The results indicated that the wine developed using *Williopsis saturnus* is found to be most preferred by the respondents.

Table 2: Mean sensory score for papaya wine with efficiency of different organisms

Sl. No	Parameter	*Saccharomyces cerevisiae*	*Lactobacillus plantarum*	*Leuconostoc oenos*	*Pediococcus cerevisiae*	*Acetobacter xylinum*	*Williopsis saturnus*
		T_1	T_2	T_3	T_4	T_5	T_6
1	Appearance	6.44	6.44	6.56	6.33	6.22	6.97
2	Colour	6.33	6.44	6.56	6.44	6.56	6.86
3	Taste	6.44	6.33	6.44	6.56	6.44	6.74
4	Aroma	6.56	6.22	6.44	6.56	6.44	6.74
5	Texture	6.33	6.44	6.33	6.22	6.22	6.41
6	Flavor	6.33	6.67	6.44	6.33	6.22	6.86
7	Bitterness	5.56	4.11	4.78	5.33	4.56	5.19
8	Strong	6.00	5.22	5.22	5.11	5.44	6.30
9	Overall Appreciation	6.44	6.56	6.56	6.56	6.33	7.09
	Mean	6.27	6.05	6.15	6.16	6.05	6.57

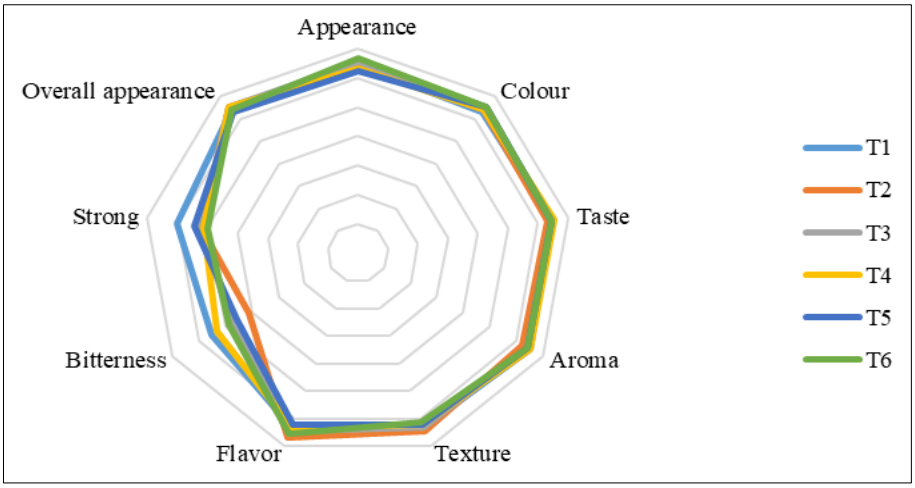

Fig 3: Mean sensory score for papaya wine with efficiency of different organisms

Numerous studies indicate that non-*Saccharomyces* yeasts are also ecologically and metabolically relevant in the wine fermentation, which has opened the door for controlled use of different yeasts in wine production. (Jolly *et al*., 2003) [19]. The results obtained in the present study regarding the superiority of *Williopsis saturnus* for fermentation and the sensory quality of the wine is similar to that reported by Trinh, *et al*., (2010). They reported that *W. saturnus* promoted metabolic connections among the yeast species during the early stages of fermentation, which enhanced the organoleptic qualities of papaya wine.

3.2 Physiochemical and proximate analysis

The fermented wine samples in Experiment 1 were subjected to physiochemical and proximate analysis and the results are presented in table 3a and 3b respectively.

In physiochemical analysis, parameters such as such as TSS, pH, Titrable acidity, Total sugar, reducing sugar, alcohol%, Specific gravity and total phenolic content of the samples were analysed.

Table 3a: Effect of pH and sugar levels on Physiochemical qualities of papaya wine

Parameters	pH 3 sugar 15 %	pH3.0 sugar 30%	pH3.0 sugar 45%	pH3.5 sugar 15%	pH3.5 sugar-30%	pH3.5 sugar-45%	pH4.0 sugar-15%	pH4.0 sugar-30%	pH4.0 sugar-45%
TSS	6.10	8.73	10.27	7.30	9.27	11.23	6.80	8.60	12.37
pH	2.46	2.91	2.63	3.23	3.14	3.31	3.56	3.87	3.64
Titrable Acidity	0.33	0.53	0.63	0.37	0.52	0.66	0.47	0.54	0.68
Total sugar	0.32	0.54	0.66	0.31	0.55	0.71	0.36	0.64	0.96
Reducing Sugar	0.23	0.44	0.57	0.29	0.65	0.60	0.27	0.51	0.84
Alcohol %	7.64	8.04	9.47	7.91	8.15	9.67	8.13	8.70	9.87
Specific gravity	0.99	1.00	0.97	0.98	1.02	0.96	0.95	1.01	1.03
Total Phenolic content	7.64	9.81	8.02	6.72	10.82	9.22	8.37	7.66	10.35

The results indicated that the wine samples developed under 45 % sugar levels and pH 4.0 recorded maximum values for TSS, pH, triable acidity, total sugar and reducing sugar, alcohol content, and total phenolic content. TSS, total sugar, reducing sugar, alcohol content was invariably influenced by the added sugar as the values for these parameters were high in samples with 45 % sugar levels.

Satav and Pethe (2016) reported that the acidic pH encourages a greater synthesis of alcohols. This may be due to an inhibition of growth of other microorganisms in an acidic pH, which increases yeasts' ability to produce alcohol. The lower alcohol percentage observed at pH 3.5 and 3.0 indicates that the fermentation activity of *Saccharomyces* might have got hindered at a more acidic pH reducing alcohol conversion.

The proximate analysis for papaya wine developed under different levels of sugar and pH are conducted and the results are presented in table 3b.

Table 3b: Effect of pH and sugar levels on nutrient contents of papaya wine

Parameters	pH 3 sugar 15 %	pH3.0 sugar 30%	pH3.0 sugar 45%	pH3.5 sugar 15%	pH3.5 sugar-30%	pH3.5 sugar-45%	pH4.0 sugar-15%	pH4.0 sugar-30%	pH4.0 sugar-45%
Vitamin A	0.25	0.30	0.34	0.29	0.37	0.41	0.42	0.33	0.61
Iron	1.01	1.08	1.12	1.14	1.02	1.20	1.23	1.19	1.24
Proteins	0.33	0.24	0.31	0.26	0.35	0.30	0.36	0.23	0.25
Carbohydrate	0.33	0.35	0.32	0.43	0.37	0.44	0.38	0.36	0.39
Fat	0.04	0.07	0.06	0.07	0.05	0.09	0.08	0.05	0.05

The results indicated that sugar level at 45% and pH 4 has recorded maximum content of Vitamin A and Iron while the other parameters did not show any conclusive results. So, it may be concluded that pH 4.0 may be ideal for developing papaya wine with better palatability, physicochemical qualities and nutrient content.

Analysis of physicochemical parameters as well as proximate analysis was also conducted for Experiment 2 in which different microbial cultures were employed for wine production. The results of the trials ae presented in table 4 a. and 4 b respectively.

Table 4a: Physiochemical analysis of papaya wine with different organisms

Parameters	*Saccharomyces cerevisiae*	*Lactobacillus plantarum*	*Leuconostoc oenos*	*Pediococcus cerevisiae*	*Acetobacter xylinum*	*Williopsis saturnus*
TSS	10.10	7.80	8.60	9.20	8.70	10.10
pH	3.70	3.40	3.63	3.50	3.60	3.67
Titrable Acidity	0.45	0.60	0.44	0.52	0.47	0.63
Total sugar	0.64	0.46	0.54	0.61	0.44	0.35
Reducing Sugar	0.44	0.37	0.45	0.54	0.32	0.26
Alcohol%	7.40	6.50	8.20	8.70	6.40	10.17
Specific gravity	0.94	1.10	1.06	0.93	0.98	0.99
Total Phenolic content	11.40	12.53	10.80	11.70	13.07	11.23

Among the various microbial cultures tried, *Williopsis saturnus* recorded significantly higher alcohol content indicating faster fermentation and reducing fermentation period. TSS, Total phenolic content, specific gravity and pH were more or less similar for *Saccharomyces cerevisiae* and *Williopsis saturnus.* However, total sugar and reducing sugar percentage were higher in *Saccharomyces* fermented wine.

Microbial cultures employed for fermentation greatly influences the flavor profile, aroma, and quality of the papaya wine. Different yeast strains exhibit unique characteristics and can contribute to the development of specific flavor profiles. Some strains are known for their ability to enhance fruity aromas, while others may produce different types of esters or volatile compounds. Trinh. *et al*., (2010) reported that W. saturnus enhanced the organoleptic qualities of papaya wine as it generates significant amounts of esters and desired volatiles, improving the fruity flavor and acceptability. Thus, selecting a suitable microbial strain that complements the papaya flavor and desired wine characteristics is crucial to evaluate the flavor profile of the resulting wines to identify the most suitable culture for papaya wine production.

Proximate analysis conducted for the vines fermented with different organisms are presented in Table 4 b. The results indicated that the wine developed by inoculation of *Williopsis saturnus* recorded maximum Vitamin A and Iron content.

Table 4b: Proximate analysis for papaya wine with efficiency of different organisms

Parameters	*Saccharomyces cerevisiae*	*Lactobacillus plantarum*	*Leuconostoc oenos*	*Pediococcus cerevisiae*	*Acetobacter xylinum*	*Williopsis saturnus*
Vitamin A	0.26	0.32	0.31	0.29	0.37	0.41
Iron	1.19	1.17	1.18	1.16	1.14	1.21
protein	0.22	0.23	0.20	0.24	0.26	0.21
Carbohydrate	0.40	0.39	0.42	0.41	0.38	0.43
Fat	0.06	0.03	0.08	0.07	0.07	0.05

3.3 Microbial analysis

Microbial analysis carried out using standard plate count method revealed that the product showed microbial growth of <10 CFU/g and fungal growth of <10 CFU/g at room temperature which is within the permissible limits after 5 days of analysis. In Experiment 1 with different levels of sugar and pH, the total hetero trophic content was low and there was no contamination from yeast and mould after 72 hours of observation. There was also no coliform count or colonies present when observed after 72 hours.

Summary and conclusion

Sugar concentration, pH, and microbial cultures play significant roles in papaya wine production. However, these factors do not act in isolation but interact with each other during fermentation. Therefore, understanding the interactions between these factors is essential for optimizing papaya wine production. Balancing sugar levels, maintaining the appropriate pH range, and selecting suitable microbial cultures are critical for achieving desired fermentation outcomes such as palatability, alcohol content, and desirable nutrient content. Conducting controlled experiments and sensory evaluations can help determine the optimal conditions for producing high-quality papaya wine.

The results of this study conclude that for production of papaya wine using *Saccharomyces cereviseae*, a sugar content of 30% and pH 4.0 will be ideal for best palatability. However, the physicochemical parameters as well as nutrient

content was best under 45% sugar and pH 4.0. Among the various organisms tried, *Williopsis saturnus* fermented wine was having the highest alcohol percentage, Vitamin A and iron content.

In conclusion, the sugar content, acidity, and pH of fruit wines significantly influence fermentation dynamics, flavor and aroma profiles, microbial stability, and overall sensory experience. Winemakers must carefully consider and optimize these parameters to achieve desirable outcomes in papaya wine production.

Overall, the development of papaya wine presents an exciting opportunity to diversify the winemaking landscape, providing consumers with a novel healthy, cost-effective fruit-based wine available in the market. Apart from diversification, wine production from papaya fruits can solve the problems of market surplus during peak seasons and related spoilage. The findings of the present study may pave the way for the commercial production and widespread availability of papaya wine, primarily benefitting the farmers and offering an enticing choice for wine enthusiasts seeking new and distinctive tasting experiences.

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