Key
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Protocol

Standard Protocol for the Production of Vegetable Wine: *Citrullus lanatus* (Thunb.) Matsum. et Nakai; *Beta vulgaris* L. and *Ipomoea batatas* L.

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Abstract

The methodology employed in this wine making process follows established scientific standards with slight modification from previous findings to develop and evaluate functional alcoholic drinks from *Citrullus lanatus* (Thunb.) Matsum. et Nakai; *Beta vulgaris* L. and *Ipomoea batatas* L. juices using fermentation with *Saccharomyces cerevisiae* var. *ellipsoideus* yeast, thereby enhancing the reliability of the results. The objective was to examine how these indigenous vegetable raw materials influence the chemical composition and sensory qualities of the resulting wines and assess their potential as value-added functional drinks. The wines were analyzed for pH, total soluble solids (TSS), alcohol content, and titratable acidity (TA), while sensory evaluation was conducted using a nine-point hedonic scale assessing colour, aroma, flavour, clarity, and overall acceptability. The resulting wines exhibited distinctive flavours, vivid colours, and health-promoting bioactive compounds, indicating strong consumer potential. Although further research is needed on factors like storage stability and environmental effects, this protocol offers a scientifically grounded baseline for new producers. Unlike traditional rice or fruit-based wines common in Manipur like "atingba," "waiyu," or "khari," wines from beetroot, watermelon, and purple sweet potato represent a novel and promising development in the region's wine industry.

Keywords: Anthocyanins**,** Betalain, Low-alcoholic beverages, Lycopene, *Saccharomyces cerevisiae,* Total soluble solids (TSS), Alcohol content, and Titratable acidity (TA), Vegetables wine.

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Graphical Overview

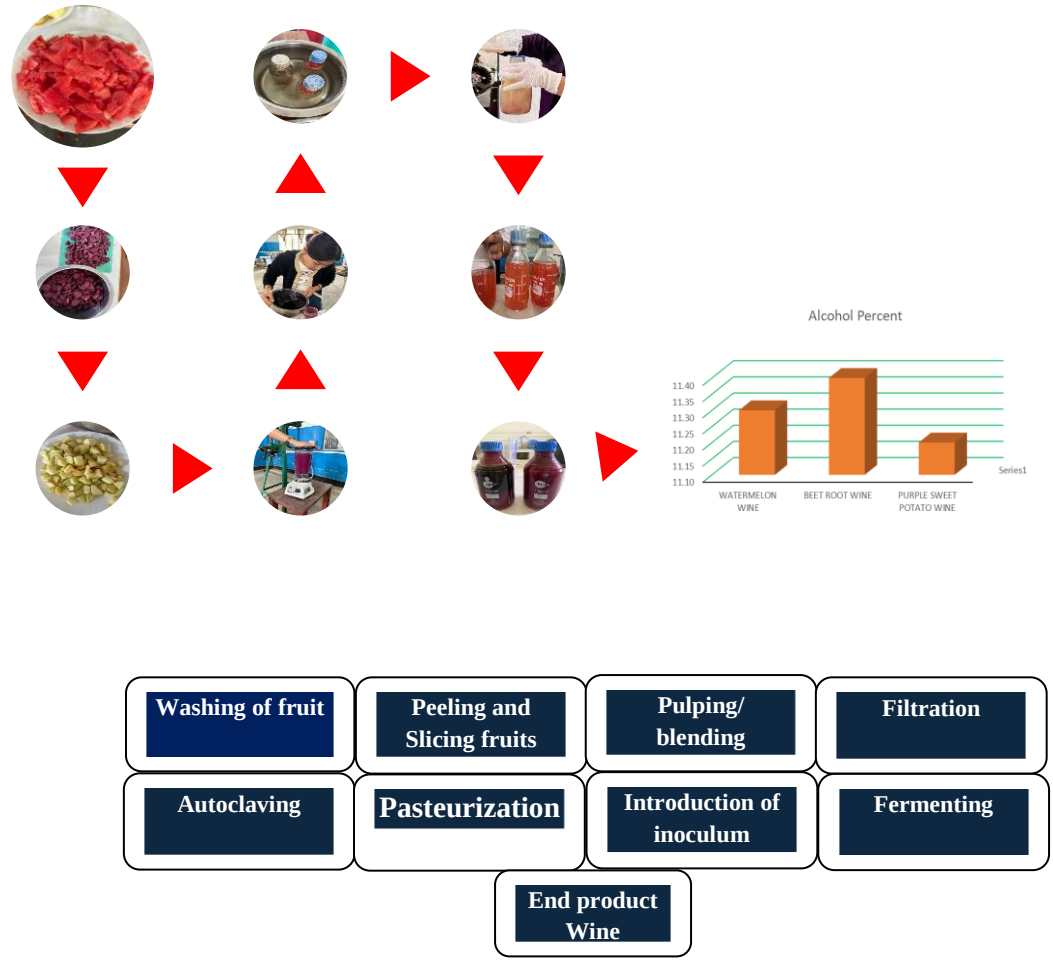

Fig 1: Standard protocol for the production of vegetable wine

1. Background and Validation of protocol

Because of its high lycopene and betalain content, watermelon and beetroot wine is renowned for its earthy aroma, vivid red color, moderate acidity, and smooth texture. Purple sweet potato wine, on the other hand, has a deeper purple color, a fruity scent, a sweeter, smoother flavor, and more antioxidants from anthocyanins. Watermelon wine is a feasible and acceptable alcoholic beverage, according to studies evaluating consumer acceptance. This is especially true when using nutrient-rich cucurbitaceous and high-volume, low-calorie "watermelon," potentially creating demand for a nourishing, revitalizing beverage (Ramirez *et al*., 2020). According to comparative research, purple sweet potato wine tends to be more widely accepted by consumers (Ray *et al.*, 2012), whereas beetroot wine appeals to those looking for strong, functional beverages (Singh *et al.*, 2021). Both offer creative, health-conscious substitutes in the wine industry and are abundant in antioxidants. These root-based wines have the potential to emerge as a unique sector in the global beverage industry as fermentation techniques advance. The majority of these veggies are grown in Manipur, although they don't keep well in storage. Considering Manipur's long history of producing wine, these potential varietals might be used to produce low-alcoholic beverages for a wider market,

which would boost our state's economy. This special section of *"research protocol"* in the key journal of MIU does not require the results or graphical statistical analysis of the study because we are presenting the specific processes involved in the wine-making process; the protocol is provided here for future reference by the scientific community. Therefore, this study aims to produce a standard procedure for the manufacturing of vegetable-based wine in order to increase consumer demand and commercialize our local product, which will be a new trend in the wine market.

1. Materials and Reagents:

This protocol was developed at the Department of Food and Technology, S. Kula Women College, Nambol. The details of experimental techniques employed during the course of present study were under the following heading.

1.1 Instruments and Equipment

- Stainless steel knives, Cutting Board, Muslin cloth, Sieve, Measuring beakers, Blender, Conical Flask, Funnel, pH meter, Hydrometer, Alcoholmeter, Refractometer, Weighing machine, Measuring cylinder, Non-absorbent cotton, Distilled water, Filter paper, Oven, Autoclave, Pipette, Burette

- For Beetroot wine:

    1. Beetroot 2kg
    2. Sugar 50g
    3. S. *cerevisiae* 5.5g
    4. Apple 1kg

- For Purple Sweet Potato
    1. Purple Sweet Potato 2kg
    2. S. *cerevisiae* 5.5g
    3. Apple 1kg
    4. Sugar 50g

- For Watermelon
    5. Watermelon 2kg
    6. S. *cerevisiae* 5.5g
    7. Apple 1kg
    8. Sugar 50g

3.2 Procurement and Collection of Ingredients

The raw materials *viz: Citrullus lanatus* (Thunb.); *Beta vulgaris* and *Ipomoea batatas*, apple (*Malus domestica),* and sugar were brought from Khwairamband Market Imphal. Brewer's yeast *(S.cerevisiae*) belongs to the brand BREW basket.

3.3 Details Procedure

The experiment was conducted during the month of April 2025 and was laid out in Completely Randomized Design (CRD). Details regarding layout is given as follow:

1. Name of the raw materials: *Citrullus lanatus* (Thunb.), Beetroot *(Beta vulgaries*), purple sweet potato (*Ipomoea batatas*), apple *(Malus domestica),* sugar, yeast (*Saccharomyces cerevisiae*).
2. Design of the experiment: CRD (Completely Randomized Design).
3. Number of Treatment: 3
4. Number of replications:3

3.4 Preparation for making wine

The raw materials *viz – Citrullus lanatus* (Thunb.), Beetroot (*Beta vulgaries*) 2Kg, purple sweet potato (*Ipomoea batatas)* 2kg, apple *(Malus domestica*)1kg, should be washed properly to remove the soil and dirt. They were cut into small pieces and blended for collecting the juices.

3.5 Beetroot and apple wine procedure

1. Preparation of beetroot and apple juice:

- Wash the vegetable and apple thorougly to remove soil, peel, and grate or chopped into small pieces and apples are washed, cored, peeled, and chopped .
- **Blender Method:** Chop the vegetable and apple into small pieces, blended with water (2 liters), and then strained through a cheesecloth to separate the pulp from the juice.

2. Addition of Additional sugar

- **Fresh Fruit:** Apple should be made into juice and should be paired with beetroot for a balanced sweetness.

3. Added sugar:

Sugar (60g) should be added before pasteurization to ensure it dissolved properly.

4. Pasteurization:

The extracted juice is poured into a sterilized vessel**.** It is then heated to 60–65°C and held for 20–30 minutes to kill spoilage microbes. After heating, the juice is cooled immediately to room temperature. Pasteurization helps stabilize the must and prevents contamination during fermentation.

5. Addition of Yeast:

Activated wine yeast **(***Saccharomyces cerevisiae***)** is added. Yeast is pre-soaked in lukewarm water for 10–15 minutes before inoculation. Yeast nutrient is also added to support fermentation. The mixture is stirred thoroughly to distribute the yeast evenly.

6. Fermentation:

The inoculated must is transferred into a clean, sterilized fermentation vessel**,** leaving about 20–25% headspace to accommodate foaming. The mouth of the vessel is either covered fitted with a fermentation lock (airlock) to allow the release of carbon dioxide while preventing entry of air. The vessel is placed in a dark, well-ventilated area and maintained at a temperature of 20–25°C**.** This primary fermentation phase lasts for 7 to 10 days**,** during which bubbling and frothing indicate active fermentation.

7.1 Fermenting Conditions and trobleshooting:

The must should be kept in an area with consistent temperature, 20 to 25°C**,** to avoid yeast stress. Containers must remain undisturbed except during daily stirring. Visible signs of fermentation include foam formation, CO_2 bubbles, and a pleasant alcoholic smell. Fermentation slows down when the bubbling subsides, usually after 7–10 days.

7. Secondary Fermentation

After primary fermentation slows, the liquid is strained to remove solids. The clear wine is transferred to a new sterile container (racking). It is sealed with an airlock and left undisturbed for 3–4 weeks**.**

8.0 Purple sweet potato and apple wine procedure

8.1 Preparation of purple and sweet potato and apple juice:

- The purple sweet potatoes should be washed thorougly to remove soil, peel, and grate or chop into small pieces and apples are washed, cored, peel,and chopped .

Blender Method: Chopped the vegetable along with apple into small pieces, blended with water (2 liters), and then strain through a cheesecloth to separate the pulp from the juice.

8.2 Addition of Additional sugar

- **Fresh Fruit:** Fresh apple should be extracted as juiced and apple juice should be paired with purple sweet potato for a balanced sweetness.
2. **Addition of sugar:** Add Sugar (60g) before the pasteurization is being done to ensure it has dissolved properly.

4. Pasteurization:

The extracted juice is poured into a **sterilized vessel**. It is then heated to 60–65°C and held for **20–30 minutes** to kill spoilage microbes. After heating, the juice is cooled immediately to room temperature. Pasteurization helps stabilize the must and prevents contamination during fermentation.

3. Addition of Yeast:

Activated wine yeast (*Saccharomyces cerevisiae*) is added. Yeast is pre-soaked in lukewarm water for 10–15 minutes before inoculation. Yeast nutrient is also added to support fermentation. The mixture is stirred thoroughly to distribute the yeast evenly.

8. Fermentation:

The inoculated must is transferred into a clean, sterilized fermentation vessel, leaving about 20–25% headspace to accommodate foaming. The mouth of the vessel is either covered fitted with a fermentation lock (airlock) to allow the release of carbon dioxide while preventing entry of air. The vessel is placed in a dark, well-ventilated area and maintained at a temperature of

20–25°C**. This** primary fermentation phase lasts for 7 to 10 days**,** during which bubbling and frothing indicate active fermentation.

9.1 Fermenting Conditions and trobleshooting:

The must should be kept in an area with consistent temperature, **20 to 25°C**, to avoid yeast stress. Containers must remain undisturbed except during daily stirring. Visible signs of fermentation include foam formation, CO_2 bubbles, and a pleasant alcoholic smell. Fermentation slows down when the bubbling subsides, usually after 7–10 days.

9.2 Secondary Fermentation:

After primary fermentation slows, the liquid is strained to remove solids. The clear wine is transferred to a new sterile container (racking). It is sealed with an airlock and left undisturbed for 3–4 weeks**.**

Fig 2. Washing, chopping, blending and straining process

Fig 3. Extraction, filteration, autoclaving, pH, TA, Alcohol percent analysis.

Fig 4. TSS and Organoleptic parameters analysis.

9. Determination of pH content

The pH of the sample should be measured using a pH meter. The meter has a sensor made up of a glass electrode and a reference electrode (usually a calomel electrode), which are connected by a potassium chloride (KCl) bridge. The meter shows the pH value based on the electrical signal produced between the electrodes. Before measuring, the pH meter was calibrated using at least two standard buffer solutions (Boulton *et al*., 1999).

10. Total soluble solids (TSS)

Total Soluble Solid should be determined by using a hand-held refractometer and expressed in percent. It is expressed in degrees Brix (°Brix) that reflects the concentration of soluble sugars and other dissolved solids (Ough & Amerin 1988). It serves as an indicator of fermentation progress and potential alcohol content. The juice should be well shaken before the drops were added to the stage of the refractometer and the result obtained.

11. Determination of Alcohol percentage

The alcohol content of the wine samples should be determined using an alcoholmeter, which measures specific gravity based on the principle that liquid density decreases with increasing alcohol concentration. Prior to analysis, the fermented wine samples were filtered to remove suspended particles that could affect accuracy. Transferred the clarified samples into a clean, dry measuring cylinder, and the alcoholmeter was carefully lowered into the liquid, ensuring it floated freely without touching the cylinder walls. The alcohol content was recorded at the meniscus level at room temperature (20 ± 1 °C). For samples measured at other temperatures, standard correction tables were applied. The results were expressed as alcohol by volume (% v/v).

12. Determination of Titratable Acidity (TA)

Pipet a 10 mL aliquot of the wine sample into a clean 250 mL conical flask. The sample should be diluted with approximately 30 mL of distilled water to facilitate titration. Two to three drops of 1% phenolphthalein indicator should be added to the diluted sample.

Titrate the solutions against standardized 0.1 N sodium hydroxide (NaOH) until a faint pink color appeared and persisted for at least 30 seconds, indicating the endpoint. The volume of NaOH consumed should be noted, and the titration should be repeated in triplicate to ensure accuracy.

The titratable acidity can be calculated using the following formula and expressed as a percentage of citric acid (A.O.A.C. 1990):

Titratable Acidity (%) = V×E×N×100÷Vs

Where:

- V = Volume of NaOH used (mL)
- N = Normality of NaOH
- E= Equivalent weight of citric acid
- Vs = Volume of wine sample taken (mL)

13. Sensory evaluation

Sensory evaluation of the wine samples was conducted to assess quality attributes such as color and appearance, aroma, clarity, flavor and taste, and overall acceptability. A panel of five semi-trained judges from S. Kula Women's College, Nambol, was selected for the evaluation. The wine samples were coded and presented in identical transparent glasses under uniform conditions to minimize bias, and the judges were instructed to rinse their mouths with water between samples to avoid carry-over effects. Each attribute was rated using a 9-point hedonic scale, where:

Specifications	Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Table 1. 9-point hedonic scale

The assessment was conducted with slight modification in accordance with standard procedure of Zhu *et al.* (2023), and based on the mean results, we utilized to interpret the wine's overall acceptance and sensory quality. Although our initial study provides an essential basis for wine production in Manipur, we advise more expert panel participation in wine testing to increase statistical significance, particularly for commercial applications. New wine producers can improve their wines and boost market viability by adding additional judges to pre-market evaluations and carrying out repeated consumer trials to determine approval. Despite its potential limitations, this baseline approach offers a protocol that is scientifically sound for future growth and improvement in Manipur's emerging wine sector.

Data analysis

Data Analysis was done by ANOVA one-way analysis using XLSTAT software (Version 2022.1). Means were compared by the Duncan's Multple Range Test. For all the data analysis, a probability level of less than 5% ($P \leq 0.05$) was considered for statistical significance.

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Competing interests: The authors declare that they have no competing interests.

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