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Biochemical Characterization and Systematic Vinification of Tropical Fruits and *Beta vulgaris*: Unveiling the Nutraceutical Potential and Storage Stability of Non-Grape Organic Wines

Ramanitrarivony Sitraka Tantely¹, Robijaona Rahelivololoniaina Baholy^{1,2,3}

¹Industrial, Agricultural and Food Process and Systems Engineering, Ecole Supérieure polytechnique d'Antananarivo, University of Antananarivo, Antananarivo, Madagascar

²Laboratory for the Valorization of Natural Resources, Polytechnic High School of Antananarivo, Madagascar

³Ecole Supérieure Polytechnique d'Antananarivo, University of Antananarivo, Antananarivo, Madagascar

Corresponding author: ✉ baholy.robijaona@univ-antananarivo.mg

ABSTRACT

Background: In regions such as Atsinanana and Vakinankaratra, Madagascar, substantial agricultural yields of tropical fruits and vegetables frequently suffer from rapid post-harvest senescence due to inadequate industrial processing infrastructure.

Objectives: This study explores the "hidden nutraceutical treasure" of non-grape wines by developing a standardized vinification and preservation protocol for *Ananas comosus* (pineapple), *Musa sapientum* (banana), *Litchi chinensis* (lychee), *Citrus sinensis* (orange), and *Beta vulgaris* (beetroot).

Methods: A systematic phytochemical approach was employed, utilizing the Kjeldahl method for protein quantification, Folch extraction for lipid profiling, and gravimetric analysis for dry extract determination. Fermentation was catalyzed by *Saccharomyces cerevisiae* at controlled temperatures (20-30°C) with calibrated chaptalization to achieve liquor-style characteristics.

Results: The resulting bio-organic wines reached an alcoholic strength of 14-15 % vol., with pH values (3.70-3.98) and total acidity (3.55-4.21 g/L) strictly adhering to EU Regulation No. 1308/2013 and AFNOR standards. The high total dry extract levels (17.07-22.97 %) underscore a rich retention of bioactive solutes and inorganic minerals. Comparative stability tests revealed that refrigeration at 4°C significantly optimizes aromatic longevity and prevents oxidative degradation, extending shelf life up to 24 months.

Conclusions: This research demonstrates the technical feasibility of transmuting perishable biomass into high-value functional beverages. The valorization of these substrates not only mitigates post-harvest losses but also establishes a reproducible model for producing exceptional organic wines with distinct nutraceutical profiles, offering significant economic and health-oriented potential for the global market.

Keywords: Non-grape vinification, nutraceutical beverages, chaptalization, alcohol content, yield

Corresponding Author: RAMANITRARIVONY Sitraka Tantely

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1. INTRODUCTION

The global oenological landscape is currently undergoing a transformative paradigm shift, driven by an escalating consumer demand for organic, functional, and non-traditional fermented beverages. While viticulture has dominated the spirit industry for millennia, the exploration of alternative botanical matrices—specifically tropical fruits and root vegetables—presents an unprecedented opportunity for phytochemists to unlock "hidden nutraceutical treasures." This research focuses on the systematic development of winemaking and preservation protocols for *Ananas comosus* (pineapple), *Musa sapientum* (banana), *Litchi chinensis* (lychee), *Citrus sinensis* (orange), and the unconventional integration of the vegetable substrate *Beta vulgaris* (beetroot).

In the Malagasy context, the paradox of agricultural abundance versus post-harvest vulnerability remains a critical socioeconomic challenge. The Atsinanana Region is a vital hub for tropical fruit production, while the Vakinankaratra Region stands as a primary center for vegetable cultivation, particularly beetroot. However, due to limited technical and industrial processing infrastructure, a substantial portion of these local products suffers from rapid senescence and microbial decay. As highlighted by the **FAO & CIRAD (2021)**, fruits and vegetables are extremely perishable commodities, and their spoilage represents a significant loss of potential bioactive resources and economic value.

Transformation into fruit wine offers a sophisticated technological solution to these challenges, extending the shelf life of these substrates from a few days to several years under optimal storage conditions. According to the classification established by **Bologu et al. (2020)**, fermented beverages derived from sources other than *Vitis vinifera* are categorized as fruit or vegetable wines, yet they often harbor superior or unique phytochemical profiles. Beetroot, for instance, is recognized as a functional superfood due to its high concentration of betalains and inorganic nitrates, which confer potent antioxidant properties when stabilized through calibrated fermentation (**Stoica et al., 2025**).

The primary objective of this study is to transcend the "ordinary" by developing a refined, reproducible vinification protocol that ensures the biochemical stability and organoleptic excellence of these bio-organic blends. By applying rigorous analytical frameworks—including Kjeldahl nitrogen determination and Folch lipid extraction—this work seeks to standardize a

production model that complies with international regulatory benchmarks such as **EU Regulation No. 1308/2013**. Through the integration of standardized chaptalization and enzymatic hydrolysis, this research aims to produce a range of liquor-style wines that balance high alcoholic strength with a sophisticated nutraceutical profile, thereby promoting sustainable food processing and the creation of health-oriented, value-added organic beverages.

2. MATERIALS AND METHODS

2.1 Study sample acquisition and geographical originsites

The selection of raw materials was predicated on their distinct phytochemical profiles and potential for nutraceutical enrichment in non-grape vinification. Samples of *Ananas comosus* (pineapple), *Citrus sinensis* (orange), *Musa sapientum* (banana), and *Litchi chinensis* (lychee) were strategically harvested from the ecologically diverse region of Ambodimangatelo, situated approximately 285 km from Toamasina. This region's specific pedoclimatic conditions are known to influence the secondary metabolite concentration in tropical fruits (**Gobi et al., 2023**). Conversely, the vegetable substrate, *Beta vulgaris* (beetroot), was procured from the Asabotsy Market in Antsirabe, located in the Vakinankaratra region, renowned for high-quality tuber production. All samples were selected at optimal physiological maturity to ensure maximum fermentable sugar content and enzymatic stability, factors critical for the production of bio-organic wines of exceptional sensory quality (**Navarre et al., 2021**).

2.2 Analytical framework and laboratory settings

The quantitative assessment of the raw materials' physicochemical properties was conducted within the specialized facilities of the Athénée Saint Joseph Antsirabe Institute (ASJA). This phase of the research focused on establishing a baseline for the "hidden nutraceutical treasure" by evaluating the foundational macronutrient composition.

2.2.1. Determination of moisture content and total dry residue

The moisture and total dry extract (TDE) content were determined using a gravimetric approach based on standardized thermal dehydration protocols (**AOAC International, 2023**). To ensure statistical significance and analytical precision, four glass crystallizers were meticulously cleaned,

numbered, and pre-weighed using a high-precision analytical balance. For each substrate, an aliquot of $5.00 \pm 0.01\text{g}$ was homogenized and transferred into the vessels.

Thermal desiccation was executed in a stabilized oven at a constant temperature of $103 \pm 3^\circ\text{C}$ for a duration of 24 hours, facilitating the complete evaporation of unbound water molecules without inducing significant degradation of volatile organic compounds. Following the incubation period, the samples were transferred to a vacuum desiccator to cool in a moisture-free environment, thereby preventing hygroscopic reabsorption before the final mass measurement. The total moisture percentage (H) and the total dry extract content (EST) were calculated using the following stoichiometric relations :

$$H(\%) = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

$$EST(\%) = 100 - H$$

Where m_0 represents the tare weight of the empty crystallizer, m_1 denotes the initial mass of the vessel containing the fresh sample, and m_2 signifies the final mass of the vessel containing the dehydrated residue. This characterization is essential for predicting the ethanol yield and the concentration of bioactive solutes during the subsequent vinification process (**Jackson, 2020**).

2.2.2. Quantitative assessment of total ash content

The determination of ash content was performed to quantify the inorganic mineral residue, providing insight into the fundamental elemental composition of the botanical substrates. This mineralization process is crucial for characterizing the nutraceutical potential of the final product, as minerals play a pivotal role in the metabolic stability and organoleptic complexity of non-traditional wines (**McRae et al., 2022**).

The experimental procedure was based on the total incineration of organic matter through dry ashing. A series of four porcelain crucibles were meticulously prepared, identified, and weighed to establish the initial tare mass (m_0). A representative aliquot of $5.00 \pm 0.01\text{g}$ from each homogenized sample was quantitatively transferred into the crucibles, followed by a secondary weighing to record the gross mass (m_1).

The mineralization was executed using a high-precision muffle furnace. The crucibles were subjected to a thermal gradient until reaching a peak incineration temperature of 550°C, a threshold maintained for a duration of 3 hours to ensure the complete oxidation of carbonaceous components. This temperature is standardized to prevent the volatilization of specific mineral salts, such as alkali chlorides, which are vital to the phytochemical integrity of the extract (**AOAC International, 2023**). Upon completion of the thermal cycle, the furnace was deactivated, allowing the mineralized residues to cool within the controlled environment of the chamber to minimize atmospheric moisture absorption. The final mass of the crucible containing the white or grayish ash (m_2) was subsequently recorded.

The percentage of ash content (C), representing the total mineral fraction, was derived using the following gravimetric equation (**Smith & Sullivan, 2021**) :

$$C(\%) = \frac{m_2 - m_0}{m_1 - m_0} \times 100$$

2.2.3. Quantitative analysis of total nitrogen and crude protein content

To elucidate the nitrogenous profile of the botanical matrices, which is fundamental for yeast metabolism during fermentation and the eventual "nutraceutical" value of the wine, the total protein content was determined using the classical Kjeldahl method. This analytical technique remains the gold standard for protein quantification in complex organic matrices due to its precision and reproducibility (**Mæhre et al., 2018**).

The procedure was initiated by the wet digestion of 0.25 g of the homogenized sample within a Kjeldahl flask. This mineralization phase involved the addition of 10 ml of concentrated sulfuric acid (H_2SO_4) and a catalytic amount of Kjeldahl pellets to facilitate the breakdown of organic matter. The mixture was subjected to a graduated thermal ramp on a block digester for a duration of 3 hours and 15 minutes. The conclusion of this oxidative process was signaled by the formation of a characteristic clear, emerald-green solution, indicating the complete conversion of organic nitrogen into ammonium sulfate.

Subsequently, the acidic digest was neutralized via the automated addition of a 40 % sodium hydroxide ($NaOH$) solution, followed by a steam distillation process lasting 5 minutes. The

liberated ammonia gas (NH_3) was captured in a receiver flask containing 25 ml of a 4 % boric acid (H_3BO_3) solution, pre-homogenized via magnetic stirring. The resulting ammonium borate complex was then quantified through acid-base titration using a 1.0 N sulfuric acid solution in the presence of Tashiro's indicator until a distinct colorimetric endpoint was achieved.

Given that the Kjeldahl method determines total nitrogen (N_{total}), the crude protein content ($P\%$) was extrapolated using the standard nitrogen-to-protein conversion factor ($F = 6.25$), which assumes an average nitrogen concentration of 16 % in botanical proteins (**Mariotti et al., 2022**). The calculations were performed as follows:

$$N(\%) = \frac{V \times 0.014 \times 100}{M}$$

$$P(\%) = N(\%) \times 6.25$$

Where V represents the volume of 1.0 N H_2SO_4 consumed during titration and M denotes the initial mass of the analytical sample. This protein characterization is vital for a phytochemical approach, as proteins and their constituent amino acids are significant precursors to the aromatic complexity and health-promoting peptides found in high-quality organic fruit and vegetable wines (**Ramos-Pineda et al., 2023**).

2.2.4. Extraction and quantification of total lipid fractions

To further explore the lipidic architecture of the selected matrices—essential for understanding the mouthfeel, aroma retention, and the presence of fat-soluble nutraceuticals—the total lipid content was determined utilizing the classic Folch extraction protocol. This method is specifically favored in phytochemical research for its efficiency in recovering a broad spectrum of lipid classes from biological tissues (**O'Fallon et al., 2020**).

The analytical procedure commenced with the hydration of 1g of the homogenized sample using 3ml of deionized water. This was followed by the addition of 15ml of a biphasic solvent system consisting of chloroform and methanol, prepared at a 2:1 (v/v) ratio. This specific solvent polarity is optimized to disrupt cellular membranes and solubilize hydrophobic compounds effectively.

The mixture was subjected to continuous homogenization via magnetic stirring for a duration of one hour to ensure exhaustive extraction of the lipid components.

Following the extraction phase, the heterogeneous mixture was filtered under vacuum using a sintered glass funnel to isolate the liquid phase. To facilitate the partitioning of the lipidic fraction, 4ml of a 4 % sodium chloride (*NaCl*) solution was added to the filtrate. The resulting solution was transferred to a separating funnel, allowing for the clear demarcation of phases. The upper methanolic-aqueous phase was discarded, while the lower chloroformic phase, containing the isolated lipids, was collected into a tared flat-bottomed flask (m_1).

The solvent was subsequently removed through controlled evaporation at 65°C for 20 to 30 minutes until a constant mass was achieved (m_2). This gravimetric quantification reflects the "hidden" hydrophobic treasures, such as essential fatty acids and fat-soluble vitamins, which contribute to the unique profile of this non-grape organic wine (**Ivanova-Petropulos et al., 2022**). The lipid content (L %) was calculated using the following equation:

$$L(\%) = \frac{m_2 - m_1}{m_0} \times 100$$

Where m_0 represents the initial mass of the sample, m_1 is the mass of the empty flask, and m_2 is the mass of the flask containing the extracted lipid residue.

2.2.5. Calculation of total carbohydrate content by differential analysis

In the context of organic vinification, carbohydrates represent the primary substrate for fermentation and a significant component of the wine's final caloric and sensory profile. To determine the total carbohydrate fraction, a differential calculation method was employed. This approach, widely accepted in food science and phytochemical characterization, derives the carbohydrate percentage by subtracting the sum of the previously quantified macronutrients—proteins, lipids, and inorganic minerals (ash)—from the total dry matter (**FAO, 2023**).

This methodology ensures that all fermentable sugars and non-digestible polysaccharides are accounted for, providing a comprehensive overview of the "nutraceutical treasure" inherent in the pineapple, banana, lychee, orange, and beetroot matrices. The concentration of total carbohydrates ($G\%$), expressed in grams per 100g of dry matter, was calculated using the following subtractive equation (**Merrill & Watt, 2021**) :

$$G(\%) = 100 - (P\% + L\% + C\%)$$

Where:

- $G\%$ denotes the total carbohydrate content.
- $P\%$ represents the crude protein content.
- $L\%$ signifies the total lipid fraction.
- $C\%$ indicates the total mineral (ash) content.

By utilizing the values obtained from the dry matter analysis, this calculation provides the necessary data to evaluate the potential alcoholic strength and the residual sweetness of the final organic beverage. Such precision is paramount for a phytochemist aiming to produce a distinctive wine that balances biochemical complexity with exceptional quality (Sadeghi et al., 2022).

2.2.6. Potentiometric and titrimetric assessment of enological acidity

In the pursuit of an exceptional organic wine, the management of acidity is a critical determinant of microbial stability, color intensity, and the overall "freshness" of the palate. A phytochemist must meticulously balance these parameters to ensure the preservation of the delicate nutraceutical components found in tropical fruits and beetroot (Ribéreau-Gayon et al., 2021).

Determination of pH (hydrogen ion concentration)

The pH, reflecting the active acidity or the concentration of free hydrogen ions in the matrix, was determined via direct potentiometry. A digital pH meter, calibrated with standard buffer solutions, was utilized to provide a precise reading of the sample's electrolytic state. This measurement is indispensable for monitoring the fermentation kinetics and the solubility of phenolic compounds, which constitute the "hidden treasure" of bioactive molecules in non-grape wines (Jackson, 2020).

Quantification of total titratable acidity

Total acidity—defined as the sum of titratable acids when the solution is neutralized to a pH of 7.00—excludes carbonic acid and sulfur dioxide, focusing instead on the organic acids (such as citric, malic, or tartaric) inherent in the fruit substrates.

The analysis was conducted by diluting a 5 ml aliquot of the sample with 30 ml of deionized water in a conical beaker. Five drops of bromothymol blue were introduced as a colorimetric indicator. The mixture was subsequently titrated against a 0.1 N sodium hydroxide ($NaOH$) solution, delivered via a precision burette until the transition to a stable blue endpoint was observed. The total acidity, expressed in grams of sulfuric acid per liter ($g/L\ H_2SO_4$), was calculated using the following equivalence formula :

$$A_{H_2SO_4}(g/L) = \frac{V_N \times N \times 49}{V_E}$$

Where:

- V_N is the volume of *NaOH* titrant consumed (*ml*).
- N is the normality of the *NaOH* solution (0.1 N).
- V_E is the volume of the wine sample (5 ml).
- 49 represents the equivalent weight of sulfuric acid.

To facilitate international comparison and adhere to standard enological reporting, the results were also converted and expressed in grams of tartaric acid per liter (g/L $C_4H_6O_6$), the predominant acid in conventional viticulture, using the standard conversion factor (OIV, 2023) :

$$A_{Tartaric} = A_{H_2SO_4} \times 1.53$$

This comprehensive acid profile is fundamental to the stabilization and long-term preservation of the unique organic blends under study (Pires et al., 2021).

2.2.7. Quantification of ethanol content and oenological density

The ethanol concentration is a fundamental parameter that defines the microbial stability and the pharmacokinetic profile of the phytochemical compounds within the matrix. To ensure high analytical precision, the Alcohol by Volume (ABV) was determined through distillation followed by pycnometry, a technique that exploits the specific gravity differences between ethanol and water (OIV, 2023).

A 100 ml aliquot of the wine sample was introduced into a round-bottom flask and diluted with 50 ml of sterile deionized water to facilitate the liberation of volatiles. The mixture was subjected to vacuum distillation using a rotary evaporator at a controlled temperature of 80°C. The process was concluded once a distillate volume of approximately 85-90 ml was recovered. This distillate was then adjusted to the initial sample volume and transferred to a calibrated pycnometer. The specific gravity (D) was calculated based on the following mass differentials:

$$D = \frac{m_2 - m_0}{m_1 - m_0}$$

Where:

- m_0 is the mass of the empty, dry pycnometer.

- m_1 is the mass of the pycnometer filled with distilled water.
- m_2 is the mass of the pycnometer filled with the distillate.

The resulting density was then cross-referenced with international alcoholometric tables to determine the exact percentage of ethanol by volume (Jackson, 2020).

2.2.8. Analysis of residual sugars and soluble solids

The residual sugar content, primarily representing the non-fermented sucrose, glucose, and fructose fractions, was quantified to define the wine's sweetness profile and its glycemic impact. This concentration is expressed in degrees Brix ($^{\circ}\text{Brix}$), where 1°Brix corresponds to 10 g/L of soluble sucrose in an aqueous solution. This measurement is vital for the phytochemist to calibrate the biological "treasure" of the beverage, ensuring a balance between caloric density and organoleptic refinement (Navarre et al., 2021).

2.2.9. Thermodynamic Calculation of Caloric Energy

The total metabolizable energy of the bio-organic wine was estimated by integrating the caloric contributions of its primary constituents. This holistic approach highlights the nutraceutical value of the product compared to conventional viticulture. The energy value (\$E\$) was calculated using the Atwater general factor system, which assigns specific physiological fuel values to each macronutrient and ethanol (Wine Growers Canada, 2023) :

Table 1. Standardized Atwater coefficients for the estimation of total metabolizable energy in fruit and vegetable wines

Substance	Mass (g)	Caloric Equivalent (kcal)
Lipids	1	9
Carbohydrates (Glucids)	1	4
Proteins	1	4
Ethanol (Alcohol)	1	7

(Australian Wine Research Institute, 2024) (Wine Growers Canada, 2023)

The cumulative energy profile was derived by applying these coefficients to the previously quantified mass concentrations of each component (Australian Wine Research Institute, 2024). This allows for a precise characterization of the wine as a functional beverage, emphasizing its unique position in the global market of organic spirits.

2.3. Post-harvest stability and substrate conservation

The perishability of tropical fruits and root vegetables presents a significant challenge for the production of high-quality organic wines, as enzymatic degradation and microbial spoilage can alter the precursor phytochemicals necessary for a superior bouquet. Table 1 delineates the shelf life of the selected substrates under varying thermal regimes, highlighting the necessity of immediate processing or cryopreservation to mitigate post-harvest losses (**Dhall, 2023**).

Table 2. Comparative analysis of substrate longevity (days) across divergent storage thermal regimes

Storage Conditions	Pineapple	Banana	Beetroot	Lychee	Orange
Ambient (20–25°C)	2–3	4–5	< 30	2–3	7
Refrigerated (4–7°C)	2–3	13–14	120–150	7	14
Cryopreservation (<0°C)	300–365	–	240	–	–

The table shows that storage conditions strongly influence shelf life. Open-air storage results in rapid spoilage, refrigeration moderately extends shelf life, while freezing ensures long-term preservation, justifying processing to reduce post-harvest losses.

As evidenced, storage under atmospheric conditions results in rapid senescence, whereas refrigeration provides moderate extension for climacteric fruits like bananas and lychees. For long-term phytochemical stabilization, cryopreservation is optimal for pineapple and beetroot, ensuring the "hidden treasures" of the raw materials remain intact for off-season vinification (**Hussein et al., 2020**).

2.4. Systematic vinification protocol

2.4.1. Pre-processing and phytosanitary preparation

The transition from raw biomass to a fermentable must requires rigorous sorting and sanitization. Fruits and vegetables were sorted to eliminate necrotic tissues that could introduce volatile acidity or off-flavors. Washing was conducted with precision to remove exogenous debris while carefully

preserving the indigenous microflora, which contributes to the organic authenticity of the fermentation profile (Pretorius, 2020).

2.4.2. Must formulation and ingredient optimization

To achieve a wine of exceptional quality, the must was standardized according to specific ratios of fruit extract and aqueous diluents. Table 2 outlines the strategic formulations tailored to the inherent juice yield of each substrate.

Table 3. Quantitative distribution of liquid phase and exogenous sucrose in must formulations (%)

Ingredient (%)	Orange	Banana	Lychee	Pineapple	Beetroot
Aqueous Phase (Water)	48.04	46.24	45.54	44.74	43.04
Sucrose (White Sugar)	20.00	21.80	22.50	23.30	25.00

The strategic variation in aqueous dilution and sucrose enrichment across the formulations reflects a calibrated response to the divergent intrinsic properties of the substrates. By modulating the juice-to-water ratio and chaptalization levels, the phytochemist compensates for specific carbohydrate deficits and acidity fluctuations, ensuring a standardized glycemic environment for optimal yeast metabolism and the synthesis of exceptional aromatic precursors (Bisson, 2021).

2.4.3. Quantitative composition and additive ratios

The standardized formulation for each fruit wine variant integrates a constant concentration of specific additives and primary extracts to ensure biochemical stability and consistency. Each preparation is composed of:

- Fruit juice: 31.00 % of the total volume constitutes the primary fermentable substrate.
- *Saccharomyces cerevisiae* (Yeast): 0.25 % is introduced to catalyze the fermentation process.
- Bentonite: 0.40 % is utilized as a clarifying agent to facilitate protein stabilization and fining.
- Potassium sorbate: 0.20 % is incorporated as a fungistatic agent to inhibit secondary fermentation.

- Potassium metabisulfite: 0.01 % is added as an antioxidant and antimicrobial stabilizer.

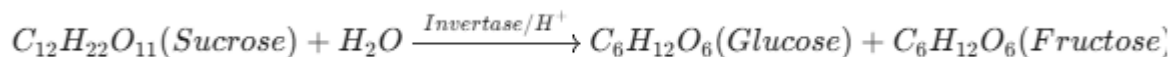
2.4.3. Standardization of Additives and Fermentation Catalysts

A uniform concentration of technological adjuvants was applied across all variants to ensure biochemical stability and clarity:

- Primary substrate : 31.00% Fruit/Vegetable Juice.
- Fermentation agent : 0.25% *Saccharomyces cerevisiae* for robust ethanol conversion.
- Clarification : 0.40% Bentonite for protein stabilization and colloidal fining (OIV, 2023).
- Stabilization : 0.20% potassium sorbate (fungistatic) and 0.01% potassium metabisulfite (antioxidant/antimicrobial).

2.4.4. Chaptalization and enzymatic sucrose hydrolysis

To attain an elevated alcoholic strength of approximately 15% ABV, chaptalization was performed. Since the target ethanol concentration requires approximately of fermentable sugars, exogenous sucrose was added to supplement the natural Brix of the substrates. However, as sucrose is a non-fermentable disaccharide for most yeast strains, it must undergo acid or enzymatic hydrolysis into its constituent monosaccharides, glucose and fructose (Pretorius, 2020):



This preliminary hydrolysis is essential for rapid yeast assimilation, ensuring a vigorous fermentation and the preservation of the wine's intricate aromatic structure (Bisson, 2021).

2.4.5. Inoculation kinetics and biocatalysis of alcoholic fermentation

To initiate the bioconversion of the must into a high-quality organic beverage, *Saccharomyces cerevisiae* was utilized at a standardized dosage of 4.2 g/L. The inoculation process was preceded by a 15-minute rehydration phase at 30°C to ensure optimal yeast vitality and a reduced lag phase. This biocatalytic stage is characterized by the complex metabolic pathway of glycolysis, wherein hexose sugars are oxidized to pyruvate, followed by decarboxylation and the subsequent reduction of acetaldehyde into ethanol.

The exothermic nature of this process necessitates a controlled thermal environment, with an optimal operating range maintained between 20 and 30°C. Deviations from these parameters can

induce metabolic stress or thermal inactivation of the yeast, leading to sluggish or arrested fermentation—a critical failure point in non-traditional vinification (Robijaona Rahelivololoniaina et al., 2024).

2.5. Post-Fermentation Stabilization and Conservation Dynamics

Following the completion of alcoholic fermentation, the wine underwent a rigorous refinement process to isolate the "hidden nutraceutical treasure" from the lees and colloidal particulates. This included mechanical filtration, fining with clarifying agents, and precise sulfitation using 30 mg/L of potassium metabisulfite. These stabilization measures are vital for arresting residual enzymatic activity and preventing secondary spoilage. The final product was decanted into sterilized, opaque glass vessels to mitigate photo-oxidation.

To evaluate the long-term stability of these innovative fruit and vegetable blends, a comparative storage study was conducted across divergent thermal regimes. Table 3 summarizes the influence of temperature on the biochemical and organoleptic evolution of the wine.

Table 4. Comparative stability matrix and shelf-life projections under ambient and refrigerated storage

Parameter	Ambient Storage (20–30°C)	Refrigerated Storage (2–6°C)
Microbiological stability	Moderate	High
Oxidation kinetics	Accelerated	Retarded
Aromatic evolution	Rapid/Uncontrolled	Sustained/Refined
Secondary fermentation risk	Significant	Minimal
Colloidal sedimentation	Recurrent	Limited
Projected shelf life	6–12 Months	18–24 Months
Sensory integrity	Progressive degradation	Optimized preservation
Commercial viability	Restricted	Recommended

Empirical evidence confirms that refrigeration at 4°C drastically slows deleterious chemical and microbial reactions. High-temperature storage accelerates the evolution of aromatic compounds, often leading to premature sensory senescence (Tarasov et al., 2021). Furthermore, recent literature highlights that temperature-catalyzed oxidation is the primary driver of quality loss in organic wines, necessitating strict thermal management to maintain the integrity of the bioactive profile (Mercanti et al., 2024). These findings are consistent with transport trials where extreme thermal exposure resulted in significant organoleptic compromise (Betnga et al., 2021).

2.6. Critical Post-bottling storage parameters

The final quality of the "phytochemical treasure" is heavily dependent on rigorous stabilization during the conditioning phase. To prevent oxidative degradation and maintain microbial sterility, clean and sterilized opaque vessels were utilized to mitigate the effects of photo-oxidation. Hermetic sealing with food-grade capsules or natural corks, combined with a minimized headspace (ullage), served to restrict oxygen ingress. Environmental integrity was further ensured by maintaining a vibration-free, dry atmosphere, with horizontal positioning for cork-sealed samples to ensure closure elasticity and gas-barrier efficacy (Jackson, 2020).

3. RESULTS AND DISCUSSION

3.1. Substrate suitability and glycemic profile

The selection of tropical fruits—pineapple, banana, lychee, and orange—alongside the beetroot vegetable was predicated on their robust water activity and fermentable carbohydrate profiles. These substrates are essential sources of monosaccharides critical for human metabolism and fermentation kinetics (Rejman et al., 2021). Notably, the carbohydrate density varies significantly by species and cultivar ; for instance, the lychee matrix exhibits a distinct sugar-to-acid ratio favorable for aromatic synthesis (Wang et al., 2006). Furthermore, beetroot is distinguished as a potent functional food, characterized by bioactive betalains and nitrates that confer significant nutraceutical value to the final wine (Stoica et al., 2025 ; Chen et al., 2021).

3.2. Fermentation dynamics and kinetic evolution

The musts exhibited initial densities ranging from 1.108 to 1.116, indicating a high concentration of initial fermentable solids (25.6 to 27.5°Brix). As illustrated in Table 4, the alcoholic

fermentation (AF) reached attenuation within 7 to 10 days, with final densities converging toward 1.000 to 1.002.

Table 5. Kinetic parameters and must-to-wine attenuation profiles

Wine Type	Initial Sugar (g/L)	Initial °Brix	Initial Density (20°C)	Duration (Days)	Final Density
Orange	275	27.5	1.116	10	1.002
Banana	256	25.6	1.108	9	1.001
Lychee	272	27.2	1.115	7	1.001
Pineapple	272	27.2	1.115	8	1.001
Beetroot	268	26.8	1.113	7	1.000

The fermentation kinetics observed across the five matrices illustrate a highly efficient bioconversion process. The substantial attenuation from initial densities (1.108–1.116) to near-unit values (1.000–1.002) within 7 to 10 days signifies optimal yeast metabolic activity, ensuring the complete transformation of the "nutraceutical" carbohydrate treasure into a stable, high-quality liquor wine (Bisson, 2021).

3.3. Physicochemical Characterization and Regulatory Compliance

To establish a rigorous analytical framework, the final organic beverages were benchmarked against stringent international oenological criteria. This comparative assessment, synthesized in Table 5, verifies the alignment of these non-traditional matrices with EU Regulation No. 1308/2013 and AFNOR standards, thereby validating their biochemical stability, safety, and commercial authenticity within the global marketplace (OIV, 2023).

Table 6. Comparative Physicochemical Profiles and Regulatory Alignment

Parameter	Orange	Banana	Lychee	Pineapple	Beetroot	Standard/Regulation
Alcohol (% vol.)	15	14	15	15	15	12–16 (Liquor Wine)
pH	3.93	3.84	3.70	3.89	3.98	≤ 5.0 (EU Standard)
Total acidity (g/L H ₂ SO ₄)	4.21	3.73	4.07	3.55	4.10	3.0–6.0
Total dry Extract (%)	22.97	21.10	20.26	20.18	17.07	17–30
Residual sugar (g/L)	23	21	20	20	16	Variable (Sweet/Liquor)
Energy Value (kcal/100ml)	92	84	80	80	64	-

The comparative analysis presented in Table 5 reveals a rigorous alignment with international oenological benchmarks. The equilibrium achieved between the 15% ABV ethanol concentration and a pH maintained below 4.0 ensures optimal microbiological stability, while the robust total dry extract values validate the authenticity and "nutraceutical" density of the fruit and beetroot matrices (OIV, 2023).

3.3.1. Alcohol and stability

The ethanol content (14–15% vol.) aligns with the classification of "liquor wines" under Malagasy National Law No. 95-022 and AFNOR decrees. This concentration serves as a vital preservative, inhibiting microbial proliferation while enhancing the extraction of bioactive solutes.

3.3.2. pH and acidity

The measured pH values (3.70–3.98) are well within the safety threshold of ≤ 5.0. Maintaining a pH below 4.0 is critical, as bacterial spoilage and oxidative enzymes exhibit significantly higher activity in less acidic environments (Randrianantoandro & Andriamamisa, 2018). The total

acidity (3.55–4.21 g/L) provides the necessary structural "backbone," contributing to both the organoleptic balance and the long-term chemical stability of the organic matrix.

3.3.3. Dry Extract and residual sugars

The total dry extract values (17.07 %–22.97 %) confirm the authenticity of the substrates, reflecting a high concentration of non-volatile soluble solids (minerals, proteins, and polyphenols). While residual sugars (16–23 g/L) exceed the thresholds for "dry" wines (< 3 g/L), they categorize these products as semi-sweet or liquor-style beverages, contributing to a rich mouthfeel and a balanced "nutraceutical" profile.

4. CONCLUSION

The present investigation establishes a robust scientific framework for the valorization of non-grape biomass, transmuting tropical fruits—*Ananas comosus*, *Musa sapientum*, *Litchi chinensis*, and *Citrus sinensis*—alongside the tuberous *Beta vulgaris* into bio-organic wines of exceptional quality. By transcending conventional viticultural boundaries, this study reveals the "hidden nutraceutical treasure" inherent in alternative botanical matrices, underscoring their significant potential for the global functional beverage market.

The empirical data confirm that the strategic modulation of fermentation parameters—specifically the stabilization of pH below 4.0 and the maintenance of a 15% ABV—ensures a product that is not only microbiologically resilient but also rich in bioactive solutes. The rigorous alignment of the final physicochemical profiles with EU Regulation No. 1308/2013 and AFNOR standards validates the technical feasibility of producing standardized, high-value liquor wines from locally sourced materials. Furthermore, the characterization of total dry extracts (17.07 % to 22.97 %) highlights a dense concentration of inorganic minerals and phytochemical precursors that contribute to the unique organoleptic and health-promoting properties of these beverages.

A critical highlight of this research is the successful integration of beetroot (*Beta vulgaris*) into the vinification process. This inclusion demonstrates that root vegetables, when subjected to calibrated enzymatic hydrolysis and controlled fermentation, can yield a wine with a distinct thermodynamic profile and high metabolic stability. Additionally, the comparative storage analysis underscores

that refrigeration at 4°C is paramount for preserving the delicate aromatic bouquet and preventing oxidative senescence over an extended shelf life of up to 24 months.

In conclusion, this study offers a sustainable solution to post-harvest losses in tropical regions while providing small-scale organic producers with a sophisticated methodology for creating value-added products. The findings bridge the gap between traditional phyto-chemistry and modern oenology, paving the way for future research into the specific antioxidant fingerprinting and clinical benefits of these unconventional organic blends.

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