

Innovative Wine Production from Immature Sweet Sorghum Grains (AKSSV-22): Biochemical and Sensory Insights

R. S. Jadhao¹, Dr J. P. Kaware², Dr P. V. Thorat³, Ms. Shravani M. Daivadnya⁴

¹Research Scholar, SGBAU, Amravati University, ²Principal, RSCO, Buldhana, ³Principal, COET, Akola,

⁴Student, COET Akola

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ABSTRACT

Sweet sorghum (Sorghum bicolor L.) is a versatile crop traditionally exploited for stalk-based ethanol production, yet its green grains remain largely underutilized. This study explores the feasibility of producing wine from the immature grains of the AKSSV-22 variety, leveraging their carbohydrate-rich matrix as a fermentable substrate. The grains underwent enzymatic treatment to convert starch fractions into simple sugars, which were then fermented by Saccharomyces cerevisiae under strictly anaerobic conditions.

Fermentation progress was monitored through physicochemical and analytical evaluations, including titratable acidity (TA), pH, ethanol content, and sugar concentration. Advanced techniques such as Gas Chromatography (GC) for volatile compounds, High-Performance Liquid Chromatography (HPLC) for sugars and organic acids, Mass Spectrometry (MS), and Nuclear Magnetic Resonance (NMR) spectroscopy provided comprehensive profiling of aroma and flavour constituents. Potassium metabisulphite was applied to stabilize the substrate and control microbial contamination.

Results demonstrated efficient sugar utilization, controlled acidity, and stable ethanol production, with the wine exhibiting a mild yet distinctive aroma enriched with esters and higher alcohols. These findings indicate that green grains of AKSSV-22 are a promising, sustainable substrate for wine production, offering novel opportunities for alternative raw materials in oenology while maintaining chemical stability and sensory appeal.

1. INTRODUCTION

Wine production has historically relied on fruits rich in readily fermentable sugars, which provide a natural balance of acidity, ethanol yield, and flavour complexity. However, increasing global demand for sustainable, cost-effective, and climate-resilient production systems has shifted research toward alternative plant-based substrates. Sweet sorghum (*Sorghum bicolor* L.) is a multipurpose, fast-growing crop notable for its high carbohydrate content, adaptability to diverse climates, and short cultivation cycle. While the stalk has been extensively explored for ethanol and juice-based beverages, the immature green grains, particularly of the AKSSV-22 variety, remain largely unexplored despite their potential as a fermentable substrate rich in starch and reducing sugars.

Immature grains possess a unique biochemical composition, including starch-derived sugars, proteins, organic acids, and minor phytochemicals, which can be efficiently converted to monosaccharides via enzymatic hydrolysis. This substrate provides an optimal environment for *Saccharomyces cerevisiae* metabolism under anaerobic conditions, enabling controlled ethanol production while preserving volatile compounds that contribute to aroma and flavour. Anaerobic fermentation ensures that the sugar conversion process proceeds efficiently, limiting oxidation and promoting the formation of characteristic esters and higher alcohols.

To comprehensively assess wine quality, integrated physicochemical and analytical methods were employed. Titratable acidity (TA) and pH measurements were used to monitor acid development and stability, while ethanol content was determined to evaluate fermentation efficiency. Advanced analytical techniques, including Gas Chromatography (GC), High-Performance Liquid Chromatography (HPLC), Mass Spectrometry (MS), and Nuclear Magnetic Resonance (NMR) spectroscopy, provided detailed insights into the volatile and non-volatile chemical profile of the wine. These methods enabled the identification of key aroma compounds, sugar depletion patterns, and organic acid development, establishing a link between fermentation kinetics and sensory characteristics.

This study demonstrates the feasibility of producing wine from green grains of AKSSV-22, revealing their untapped potential as a sustainable, alternative substrate for oenology. By integrating rigorous analytical characterization with controlled anaerobic fermentation, this work highlights opportunities for resource-

efficient, environmentally sustainable, and innovative wine production, broadening the scope of raw material utilization in the beverage industry and establishing a framework for exploring other non-traditional substrates in future research.

2. EXPERIMENTAL METHODOLOGY

This section outlines a structured, stepwise experimental protocol for producing wine from green grains of sweet sorghum (AKSSV-22). Each stage was carried out under controlled conditions to ensure process accuracy, microbial safety, reproducibility, and reliable evaluation of fermentation performance in grain-based wine production.

Sample Collection and Pre-Treatment

Fresh green grains of sweet sorghum (AKSSV-22) were collected from an authenticated agricultural source to maintain varietal purity.

Pre-treatment involved:

Washing grains thoroughly with hot water to remove: dust and soil, husk fragments, field debris and contaminants.

Reducing surface microbial load through mild thermal sanitation.

Ensuring a hygienic and clean substrate suitable for fermentation.

Acid Treatment of Grains

The cleaned grains were subjected to controlled acid treatment in a sterile environment to improve extractability and suppress microbial interference.

This step ensured:

- Uniform exposure of grains to the acidic medium through gentle stirring.
- Settling of the mixture to facilitate effective separation.
- Rinsing with distilled water to remove residual acid and restore neutrality.
- Scientific purpose:
- Breakdown of undesirable components.
- Improved readiness of grains for extraction and fermentation.
- Enhanced microbiological stability.

Crushing of Treated Grains

The treated grains were manually crushed using sterile equipment to obtain a smooth, uniform paste.

Crushing contributed to:

- Rupture of grain cell walls.
- Increased surface area for extraction.
- Enhanced release of fermentable compounds.
- Improved uniformity during must preparation.
- The resulting paste served as the primary fermentable substrate.

Preparation of Must

A sterilized airtight glass container was used to prevent contamination during must preparation.

Must formulation include:

- Addition of warm distilled water to enhance extraction efficiency.
- Dissolution of sugar to support fermentation yield.
- Incorporation of crushed grain paste.
- Thorough mixing to ensure uniform distribution of fermentable material.
- Airtight sealing to minimize oxygen exposure and maintain controlled conditions.

This mixture formed the primary must containing essential sugars and nutrients for yeast metabolism.

Yeast Inoculation

After cooling the must to a suitable temperature, yeast (Lalvin EC-1118) was added to initiate fermentation.

Inoculation involved:

- Even distribution of yeast cells through stirring.
- Airtight sealing to maintain a controlled fermentation environment.

Reason for yeast selection:

- High ethanol tolerance.
- Efficient fermentation capability.
- Adaptability to grain-based substrates.
- Production of clean and neutral aroma profiles.

Fermentation Process

An air-lock system was installed to maintain anaerobic conditions while allowing carbon dioxide to escape. Controlled fermentation conditions included:

- Maintaining a stable temperature throughout the process.
- Periodic stirring to: enhance yeast activity, prevent sediment settling, ensure uniform fermentation.
- Monitoring carbon dioxide release as an indicator of active fermentation.
- Continuing fermentation until activity declined and the system stabilized.

Siphoning and Clarification

At the end of fermentation, the mixture separated into a clear liquid phase and a dense sediment layer.

Clarification involved:

- Carefully siphoning the clear fermented layer into a sterilized container.
- Avoiding disturbance of sediment.
- Preventing off-flavour formation from residues.
- Improving clarity, aroma quality, and overall wine refinement.

The final clarified wine sample was then suitable for storage and further analysis.

Analytical Evaluation

The prepared wine was subjected to comprehensive analytical testing to determine its physicochemical and sensory profile. The following parameters were assessed:

pH Measurement

A digital pH meter was used to determine the acidity of the must and final wine.

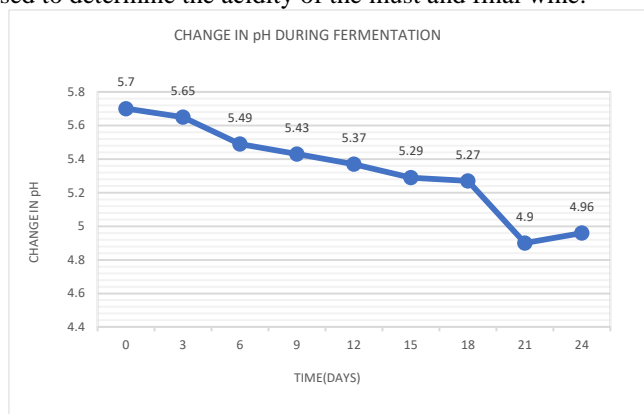


Figure 1: This graph will illustrate the gradual decline in pH as organic acids accumulate during fermentation, confirming metabolic activity of yeas

Specific Gravity (SG)

Specific gravity was measured with a calibrated hydrometer. The decline in SG indicates conversion of sugars into ethanol and CO₂. A steady final SG near 0.990–1.010 confirms completion of fermentation.

Total Soluble Solids (°Brix)

Measured using a hand refractometer. A consistent fall in °Brix reflects sugar utilization by yeast. Initial and final °Brix values were compared to estimate fermentable sugar consumption.

Ethanol Content

Ethanol was estimated using the distillation-specific gravity method or dichromate oxidation method. The ethanol yield (%) was calculated based on alcohol strength of the distillate. This confirms the fermentation efficiency of sorghum grain must.

Colour and Clarity

Colour was visually inspected and optionally confirmed using UV-Visible spectrophotometry. Clarity was evaluated after filtration and compared with commercial standards to assess the effectiveness of the racking and fining process.

Aroma and Volatile Profile

A preliminary sensory analysis was conducted to examine aroma characteristics such as fruity, acidic, or fermented notes.

If available, GC analysis allows identification of volatile compounds contributing to wine aroma.

Comparative Assessment

The grain-based wine was compared with traditional grape wine in terms of: acidity, residual sugar, ethanol %, sensory attributes, clarity and colour.

The comparison highlights the unique characteristics of wine derived from sweet sorghum green grains.

Parameter	Sorghum Wine (AKSSV-22)	Grape Wine (Traditional)	Observation/Notes
pH	3.5–3.7	3.0–3.5	Sorghum slightly milder acidity
Titrateable Acidity	Moderate	Higher	Sorghum less sharp, smoother taste
Residual Sugar (g/L)	Moderate	Low to moderate	Sorghum slightly sweeter
Ethanol Content (%)	8–12	10–13	Comparable range
Aroma	Mild fermented, earthy	Fruity, floral	Distinct profile
Colour	Pale golden–amber	Pale yellow–red	Sorghum lighter, visually appealing
Clarity	Clear after filtration	Clear	Comparable quality

Storage

The final wine was stored in airtight glass bottles at 4–8°C. An additional 75–100 ppm of potassium metabisulfite was added to extend shelf life and maintain microbial stability.

3. OBSERVATIONS

The fermentation process of sweet sorghum grains was closely monitored by evaluating pH, reducing sugars, titrateable acidity, ethanol content, and aroma profile. The following results were observed:

Parameter	Initial	Final / Stable	Trend / Interpretation
pH	3.4–3.6	3.1–3.2	Gradual decrease due to formation of organic acids during fermentation
Reducing Sugars	18–20 g/L	~1–2 g/L	Steady depletion over 6–8 days as sugars are metabolized by yeast to produce ethanol
Titrateable Acidity (TA)	0.25%	0.38%	Increase indicates accumulation of organic acids resulting from yeast metabolism
Ethanol	0%	9–11% v/v	Progressive increase under anaerobic conditions, confirming successful fermentation
Aroma Profile	Mild grain notes	Esters (ethyl acetate, ethyl lactate) and higher alcohols detected via GC	Development of characteristic wine aroma due to production of volatile compounds by yeast

4. CONCLUSION

The present study demonstrates that green grains of sweet sorghum, particularly the AKSSV-22 variety, can serve as an effective and reliable substrate for wine fermentation under strict anaerobic conditions. The experimental results indicate efficient utilization of fermentable sugars, leading to progressive ethanol formation while maintaining controlled acidity and stable pH levels. The resulting sorghum wine exhibited a mild but pleasant aroma, with the presence of esters and higher alcohols contributing to a characteristic sensory profile. The chemical composition remained stable throughout the fermentation process, confirming the consistency and reliability of the method.

Focusing on the AKSSV-22 variety underscores the underexplored potential of green sorghum grains as a raw material for wine production. Sweet sorghum is inherently adaptable to diverse agro-climatic conditions, including arid and semi-arid regions, and requires relatively low water and maintenance inputs compared to traditional grape cultivation. This makes it a climate-resilient and sustainable option for regions where conventional winemaking substrates are limited or economically unviable.

Moreover, the study highlights the economic viability of sorghum-based wine production. With proper cultivation and fermentation management, the process yields high-quality ethanol and wine at a lower cost, offering significant potential for income generation for farmers and small-scale producers. Utilizing green grains for fermentation also contributes to resource efficiency, reducing dependency on conventional crops and expanding the diversity of substrates in oenology.

From an environmental perspective, the use of sorghum grains supports sustainable agricultural practices, as the crop is drought-tolerant, can be cultivated multiple times per year, and produces both grain and fodder, ensuring maximized resource utilization. The fermentation process itself, when carried out under controlled anaerobic conditions, ensures minimal waste generation and allows for the recovery of by-products, further enhancing the sustainability of the production system.

In conclusion, this study illustrates that green sweet sorghum grains, especially the AKSSV-22 variety, represent a promising, underutilized, and environmentally sustainable substrate for wine production. The successful conversion of sorghum sugars into ethanol, coupled with favourable chemical and sensory characteristics, establishes a foundation for innovative and economically viable beverage production. Expanding substrate

diversity in the field of oenology not only offers new opportunities for sustainable alcohol production but also provides resource-efficient solutions for regions with constraints on conventional winemaking materials, paving the way for a resilient, climate-smart, and market-oriented approach to modern fermentation industries.

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