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Integrated Upstream and Downstream Processing of Sorghum-Based Beer Fermentation Using Saccharomyces cerevisiae with Honey as a Natural Flavor Enhancer

Dhirendra Singh[1], Rashi Srivastava[2], Shivanshi Tripathi[3]

[1,2,3]Assistant Professor, Department of Biotechnology Engineering, Faculty of Engineering & Technology, Khwaja Moinuddin Chishti Language University. Lucknow, 226013, Uttar Pradesh, India

dheerendra.singh301@gmail.com, rashisrivastava012@gmail.com, shivanshit58@gmail.com

A B S T R A C T :

Sorghum (*Sorghum bicolor*) is an important cereal crop widely utilized for food, feed, and industrial applications, with growing interest in its use as a raw material for brewing due to its gluten-free nature and adaptability to diverse environments. This study aimed to evaluate the integrated upstream and downstream processing of sorghum-based beer production using *Saccharomyces cerevisiae* as the fermenting organism and honey as a natural flavouring agent. Critical upstream parameters, including malting efficiency, mashing performance, sugar release, and fermentation kinetics, were systematically assessed to determine their impact on product quality. Downstream processes, including clarification, stabilization, and flavour retention, were optimized to ensure sensory acceptability and product stability. The incorporation of honey enhanced both aroma and taste while maintaining fermentation efficiency, thereby improving overall product value. In addition to demonstrating the potential of sorghum as a sustainable brewing substrate, the study emphasizes the role of honey as a functional additive that supports natural flavour enrichment without synthetic enhancers. The findings also underline the economic significance of sorghum brewing in promoting local raw material utilization and reducing dependence on conventional barley-based processes. Furthermore, honey incorporation not only adds functional properties but also aligns with consumer demand for naturally flavoured, healthier alternatives. By integrating bioprocess optimization with sustainability and market-driven innovation, this study provides a framework for developing novel, regionally adapted beer products that combine nutritional benefits, cultural relevance, and environmental responsibility.

Keywords: Sorghum, *Saccharomyces cerevisiae*, Beer production, Upstream processing, Downstream processing, Honey flavouring, Fermentation efficiency, Sustainable brewing

1. Introduction

Sorghum (*Sorghum bicolor*), a drought-tolerant and nutrient-rich cereal crop, has gained significant attention as an alternative substrate in brewing, particularly in regions where barley cultivation is limited or gluten-free options are in demand. Globally, sorghum ranks among the top five cereal crops and plays a crucial role in food security, livestock feed, and industrial applications (Ciocan et al., 2023). Its adaptability to marginal soils and resistance to water stress make it an ideal raw material for sustainable brewing processes in developing and emerging economies. With increasing consumer awareness of both dietary preferences and sustainability, the exploration of sorghum as a brewing substrate provides both technological opportunities and socio-economic benefits (Tan et al., 2023). Unlike barley, sorghum possesses unique starch and protein structures that influence brewing outcomes. Its starch granules are generally smaller and exhibit higher gelatinization temperatures, ranging from 72–82 °C compared to 62–65 °C in barley (Tan et al., 2023). This creates challenges in enzymatic hydrolysis during mashing, often requiring either exogenous enzyme supplementation or optimized malting practices to improve conversion efficiency. Sorghum also has comparatively lower amylase activity, which reduces the breakdown of starch into fermentable sugars (Ciocan et al., 2023). Despite these limitations, sorghum contains high levels of α-glucosidase, which can enhance fermentability when malting and mashing conditions are controlled appropriately (Tan et al., 2023). These characteristics make sorghum both a challenge and an opportunity for beer production, necessitating integrated upstream processing strategies to maximize sugar yield. From a nutritional perspective, sorghum is gluten-free, making it highly attractive for brewing beers targeted at individuals with celiac disease or gluten intolerance (Budner et al., 2024). The development of gluten-free beers from sorghum aligns with global dietary shifts towards allergen-free foods, enhancing the commercial value of sorghum brewing (Ciocan et al., 2023). The choice of yeast is another critical factor in sorghum beer production. *Saccharomyces cerevisiae* has been widely adopted in both traditional and industrial sorghum fermentation due to its ethanol tolerance, rapid fermentation kinetics, and desirable flavor compound production (Budner et al., 2024). Yeast strain variability directly affects ester and higher alcohol formation, which determine aroma, mouthfeel, and consumer acceptance. Studies on sorghum beers produced with pure cultures of *S. cerevisiae* compared to mixed or wild cultures indicate that controlled fermentation yields more consistent flavor and improved stability (Volatile compounds of traditional sorghum beer, 2021).

Additionally, yeast metabolism during fermentation is influenced by wort composition, oxygen availability, and the presence of supplementary sugars. In sorghum wort, the limited maltose content can restrict fermentation efficiency; however, supplementation with alternative sugar sources or co-fermentation strategies can improve ethanol yield and flavor complexity (Macharia et al., 2022). This presents an opportunity to introduce natural sweeteners such as honey, which not only provide fermentable substrates but also contribute aromatic compounds (Cicha-Wojciechowicz et al., 2024).

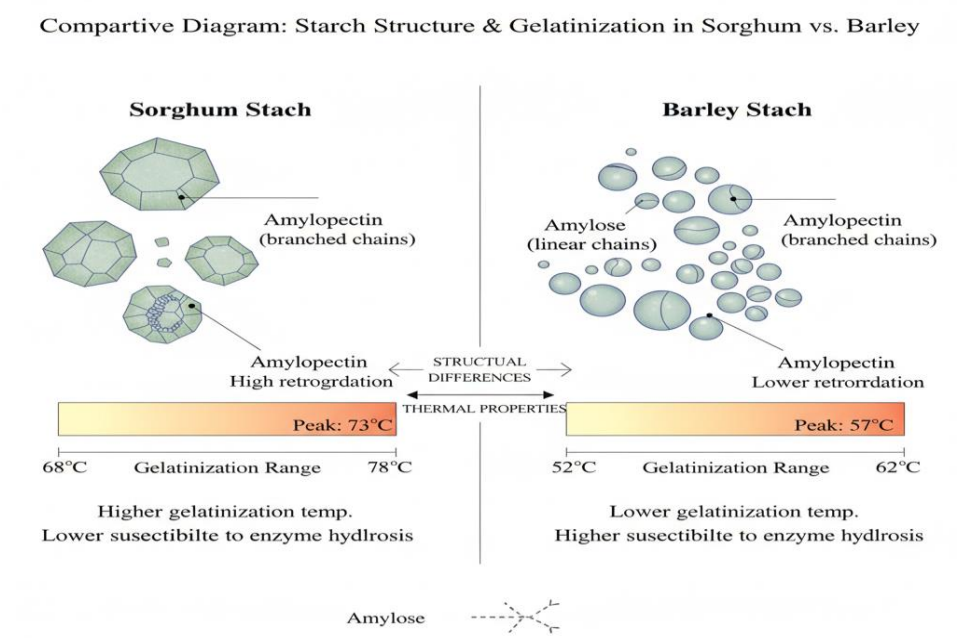

Fig.1: Starch structure and gelatinization in sorghum and barley

Honey as a Natural Flavor Enhancer

Honey has been used traditionally in mead production and increasingly in experimental beers due to its rich composition of simple sugars, polyphenols, and volatile organic compounds (Cicha-Wojciechowicz et al., 2024). Its incorporation in fermentation provides several benefits: it enhances aroma, balances acidity, and contributes to residual sweetness depending on fermentation conditions. Honey also exhibits antimicrobial and antioxidant properties, which may support microbial stability and shelf life of the final product (Mukisa et al., 2017). In the context of sorghum beer, honey addition could overcome limitations in sugar availability while simultaneously improving sensory quality. Mukisa et al. (2017) demonstrated that in the production of *Enturire*, a traditional sorghum-based alcoholic beverage, honey accelerated acidification and enhanced the aroma profile. Similarly, Cicha-Wojciechowicz et al. (2024) found that honey variety and timing of addition significantly influenced odor-active compounds in meads, suggesting its potential application in beer brewing for tailored flavor outcomes.

Upstream and Downstream Processing Integration

Upstream processing in brewing refers to the sequence of steps from grain preparation to wort fermentation, including malting, mashing, and yeast propagation. For sorghum, optimizing malting is essential to activate hydrolytic enzymes while minimizing anti-nutritional factors such as tannins and phytates (Tan et al., 2023). Mashing strategies often include temperature adjustments or exogenous enzyme additions to ensure complete saccharification. The integration of honey at this stage can serve as a supplementary sugar source, potentially improving wort fermentability and reducing lag phases during fermentation (Cicha-Wojciechowicz et al., 2024). Downstream processing, on the other hand, ensures the stability and quality of the final product. This includes clarification, stabilization, carbonation, and packaging. For honey-flavored sorghum beers, downstream considerations are particularly important because volatile aroma compounds derived from honey are sensitive to oxidation, filtration, and thermal treatments (Budner et al., 2024). Clarification processes must therefore be carefully controlled to retain flavor compounds while achieving the desired clarity and microbial safety.

The use of sorghum and honey in brewing also aligns with broader sustainability goals. Sorghum requires less water and agrochemical inputs compared to barley, making it environmentally resilient (Ciocan et al., 2023). Honey, as a natural and locally sourced sweetener, supports apiculture and rural economies while reducing reliance on synthetic flavoring agents (Mukisa et al., 2017). Together, their integration into beer production promotes local resource utilization, circular economy practices, and reduced carbon footprints. Moreover, the development of sorghum-based honey-flavored beers supports diversification in brewing industries, offering niche products that appeal to health-conscious and eco-aware consumers. With rising competition in the global beer market, innovations in raw materials and flavoring strategies are essential for maintaining consumer engagement (Budner et al., 2024). The present study therefore seeks to systematically evaluate integrated upstream and downstream processes in sorghum beer brewing using *S. cerevisiae* with honey as a natural flavor enhancer. Specifically, it aims to (i) optimize malting and mashing parameters for sorghum, (ii) assess fermentation kinetics and flavor compound development with *S. cerevisiae*, (iii) evaluate the influence of honey addition on sensory quality, and (iv) establish downstream processing conditions that maximize flavor retention and product stability. The integration of these aspects is expected to provide a holistic process model that bridges traditional brewing knowledge with modern biotechnological innovations.

2. Methodology

Pretreatment of Sorghum

Malting is the process where the sorghum grain is made ready for brewing. Malting is broken down into three steps, which help to release the starches in the sorghum. First, during steeping, the grain is added to a vat with water and allowed to soak for approximately 40 hrs. During germination, the grain is spread out on the floor of the germination room for around 5 days.

a. Steeping

Steeping is the process of soaking grains into water to soften the grains for germination. The sorghum seeds are soaked into water (1kg sorghum grains into 4 L of water) and 10mL/L 90% ethanol. The 90% ethanol helps in removal of rigidity of sorghum seeds. The soaking is done for 2 to 3 days.

b. Germination of sorghum grains

For germination of sorghum grains, the steeped grains are put into 96% humid condition for 5 days. Germination process releases several starches converting enzyme like amylase. These enzymes convert starch (non - fermentable sugar) into glucose, di saccharides & tri saccharides (Fermentable sugars).

c. Kilning

Kilning is the heating of germinated sorghum to dry it and develop malty, biscuit-like flavours. The largest portion of malt in most beers today is pale malt that is only gently dried at relatively low heat to preserve the integrity of its enzymes. Kilning is the final stage in traditional malting, after steeping and germinating, and its techniques and equipment have been developed over many centuries. The kilning process is fairly simple, but its chemistry is complex. Kilning is invariably done in two or three stages. Initially, most of the surface moisture of the germinated grain is driven off. At the final stage, the malt is "cured." The goal is to reduce the grain's moisture content from about 40% to 50% down to at least 4% to 6%. Different maltsters use different temperatures and time intervals for the different kilning phases. A typical sequence is step one at 50°C to 60°C (122°F to 140°F), step two at 65°C to 75°C (149°F to 167°F), and a curing step at 80°C to 105°C (176°F to 221°F). The sequencing of the temperature levels is important. If the grain is heated too moist at too high a temperature, its enzymes would be denatured and thus it would be rendered useless for mashing. The kilning process was done by using hot air oven.

d. Milling

The beer brewing process begins with the milling of the grain. The idea here is to increase the exposed surface area of the sorghum malt - similar to the process of grinding coffee beans before making coffee. The starches are then extracted by soaking the milled grain, or grist, in hot water. Too course a grind will result in an incomplete extraction of the starches. Too find a grind may result in the grain forming a thick cake that does not allow the liquid to drain easily. This lack of drainage is called a stuck mash. Sometimes additional husks (also called hulls) are added to the grain to promote proper drainage. Milling the sorghum too finely also damages the husks, which act as a filter bed when draining liquid from the crushed grains. These damaged husks will release tannins, which will give an undesirable astringency to the finished beer.

Mashing

The next stage in brewing after milling the grain is mashing, which involves soaking the crushed malt in hot water. In brewing terminology, water is often referred to as "liquor," but for clarity, the term water will be used here. Mashing serves two main purposes: it releases starches from the grain into the liquid and activates naturally occurring enzymes that convert these starches into fermentable sugars. A helpful analogy is steeping a tea bag—except in this case, the extract is called "wort" (pronounced *wert*). Yeast cannot directly metabolize starch, but they efficiently utilize simpler sugars. Malted barley is commonly chosen for brewing because it contains enzymes, primarily amylases, which drive the conversion of starch into sugars. The mash temperature is critical since it determines enzyme activity and, consequently, the balance of fermentable and unfermentable sugars produced. Two major forms of the enzyme amylase are active during mashing: alpha-amylase and beta-amylase. At mash temperatures around 70–80 °C, alpha-amylase predominates. While effective, it tends to leave behind unfermentable sugars, resulting in a beer with lower alcohol content but a fuller mouthfeel. Conversely, lower mash temperatures favour beta-amylase, which provides more thorough starch conversion and leads to higher alcohol yields with a lighter body. When brewing with adjunct grains, higher mash temperatures are often required, so these grains are usually processed first before cooling the mash and adding barley. Another approach is decoction mashing, a traditional German technique still used in some classic beers like *Pilsner Urquell*. In this method, a portion of the mash (both grain and liquid) is removed, boiled to promote flavour extraction and sugar caramelization, and then returned to the mash tun to raise the overall mash temperature.

The powdered sorghum is added into distilled water (1200 grams sorghum into 4 L of distilled water) and this mixture is boiled at 70 degrees C for 20 minutes. After this cycle, the temperature is raised to 92-degree C. After this mixture is allowed to cool down and then wort is separated by using sieving clothe.

MRS Broth Preparation and Inoculum Transfer

The MRS formulation was developed by de Man, Rogosa and Sharpe.

Enzymatic digest of animal tissue (10 g), beef extract (10 g), yeast extract (5 g), dextrose (20 g), NaC2H3O2 (5 g), polysorbate 80 (1 g), KH2PO4 (2 g), ammonium citrate (2 g), MgSO4 (0.1 g), MnSO4 (0.05 g), per 1000 mL, pH 6.5.

After adding all chemicals, the sterilization process was done by using autoclave at standard condition. After autoclaving, the MRS broth is allowed to cool down to room temperature. After this, MRS medium broth and Slant of *S. cerevisiae* were transferred into LAF cabinet. By using Standard Operation Procedure, the inoculums were transferred into MRS broth and the flask containing MRS broth and inoculums was incubated in Orbital shaker cum incubator at 25 degree C for 72 hours.

Inoculum transfer into Samples

After 72 hrs, The MRS broth flask along with all the samples were transferred into LAF. Firstly about 10 mL of honey is added into sample 3. After this, 50 mL of cultured samples was added into each sample by following Standard Operation Protocol of LAF cabinet. After this the cotton plug were replaced by the balloons to check the gas production.

The Fermentation Process

The flasks are then incubated at 25 degree C for fermentation process. The whole fermentation process for beer production took 7 days. After 72 hrs, the sample of gas was taken from balloon by using sterile syringe for estimation of production of CO2.

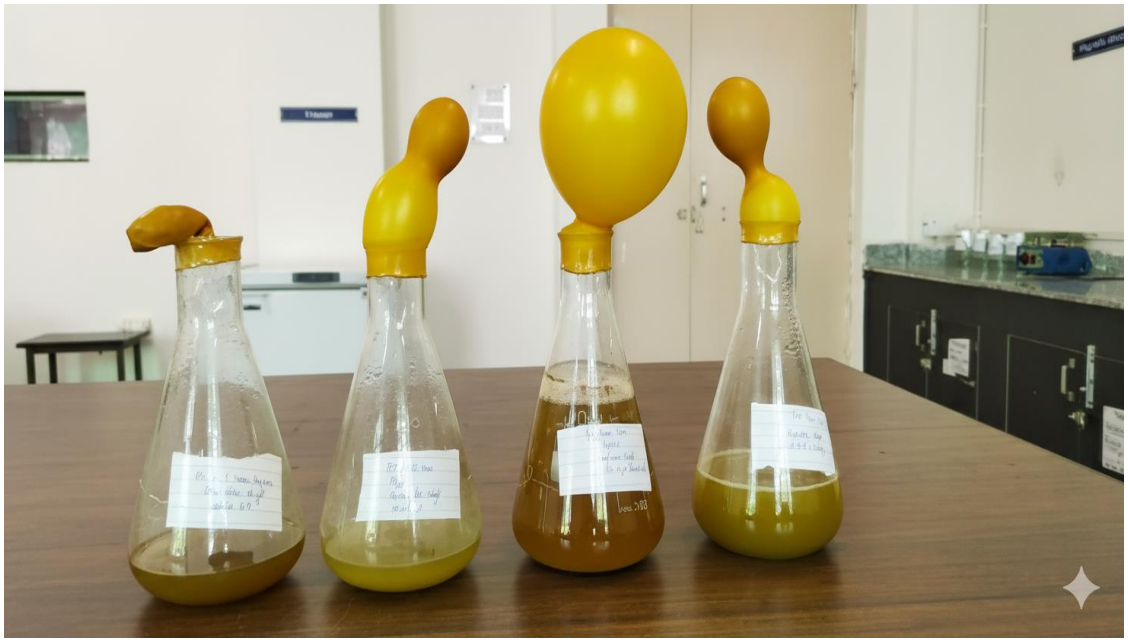

Fig.2: Fermentation process (After 72 hours)

Confirmatory Test

a. Change in Aroma

This test was based on smelling. As humans can record specific aroma, hence when next time they encountered same aroma, they recognize it. After 7 days, firstly we smell our samples, then we brought market beer sample to check aroma of our samples is similar or not. After this, the samples were taken to those persons who intake beers to check our samples are having similar aroma or not.

b. CO_2 Estimation by Gas Chromatography

A gas sample was collected from a balloon using a syringe and subsequently transferred to the Gas Chromatography (GC) system. Before analysis, the instrument was first run with a blank to confirm proper functioning and establish a baseline. The collected sample was then introduced into the injection port of the GC, where separation and detection of gases were carried out following standard operational procedures. Hydrogen was employed as the carrier gas to facilitate the movement of analytes through the column. Upon completion of the analysis, the system generated a chromatogram along with associated data, which was used to quantify the concentration of carbon dioxide present in the sample. Thus, CO_2 estimation was successfully achieved using Gas Chromatography.

c. Alcohol Estimation by Hydrometer

A hydrometer is a device used to measure the relative density of liquids, functioning on the principle of buoyancy. It is generally constructed from a sealed glass tube that has a broadened base for flotation, a weighted ballast for balance, and a slender stem marked with measurement scales. During use, the test liquid is placed in a tall container, such as a graduated cylinder, and the hydrometer is carefully lowered until it floats freely. The reading is taken at the point where the liquid surface meets the stem, which reflects the liquid's density or specific gravity. Depending on the calibration, different hydrometers serve specialized purposes: a lactometer is used for milk density, a saccharometer for sugar concentration in solutions, and an alcoholometer for determining alcohol content in beverages. By correlating density with concentration, a hydrometer provides a simple and effective method for alcohol estimation.

Calculation

For an approximate estimation, the number is 131.25. Percent alcohol can be given by the formula:

ABV(%) = (Initial Gravity - Final Gravity) * 131.25.

**The intial specific gravity of was taken just after pH adjustment.

d. Glucose Estimation by DNS Method Using Spectrophotometer

The 3,5-Dinitrosalicylic acid (DNS) method is a widely used colorimetric assay for estimating glucose and other reducing sugars. DNSA (IUPAC name: 2-hydroxy-3,5-dinitrobenzoic acid) reacts with reducing sugars under alkaline conditions to form 3-amino-5-nitrosalicylic acid, a compound with strong absorbance at 540 nm. The reaction depends on the presence of a free carbonyl group (C=O), which is characteristic of reducing sugars. In this process, the aldehyde group of glucose (or the ketone group of fructose) undergoes oxidation, while DNSA itself is reduced.

Reagent Preparation

1. Prepare 20 mL of 2N sodium hydroxide (NaOH).
2. Dissolve 1 g of DNS in the NaOH solution with continuous stirring.
3. Separately, dissolve 30 g of sodium potassium tartrate in 50 mL of distilled water.
4. Gradually add the tartarate solution to the DNS–NaOH mixture while stirring.
5. Adjust the final volume to 100 mL with distilled water.
6. Store the prepared reagent in a brown bottle at 4 °C. Filter if necessary.

Procedure

1. Label five test tubes as Blank, 1, 2, 3, and 4.
2. Prepare glucose standards of known concentrations and dispense into tubes 1–4. The Blank contains only distilled water.
3. Add 3 mL of DNS reagent to each tube.
4. Mix thoroughly and place the tubes in a boiling water bath for 15 minutes to allow color development.
5. Cool the tubes to room temperature using a cold water bath.
6. Measure absorbance at 540 nm using a spectrophotometer.
    - First, set the instrument to zero using the Blank.
    - Then record absorbance values of standards (Tubes 1–4).
    - Ensure the cuvette is rinsed after each reading.
2. Plot a standard curve of absorbance versus glucose concentration.
3. Measure the optical density (OD) of the beer samples under the same conditions.
4. Using the standard curve, determine the glucose concentration in the final beer samples.

e. Microbiological Testing of Beer Sample

After fermentation process the beer samples was centrifuged at 3338 X g to remove microbial cells. After centrifugation, the supernatant (beer) is separated from pellet inside laminar air flow cabinet. After this, plates of nutrient agar medium were made and 100 micro-L samples from sample 2,3 and 4 were poured onto the plate and then the plates are incubated at 25 degree C for 48 hours. After this colony is counted by using colony counter equipment. Sample 1 on pH 2, having no growth, hence need not to check.

Results

As aroma can't be recorded in terms of number, hence it can only be smell. As humans can record specific aroma, hence when next time they encountered same aroma, they recognize it. This was the concept of this test. First we smell manufactured beers of different brands, then we smell our own samples. As there was no growth in sample 1, hence it was the nearly same smell as wort. Sample 2 which was our control, had nearly similar aroma as beer of different brands. The sample 3 in which honey was added, had a unpleasant aroma and its aroma was not similar to beer. Sample 4 had aroma of beer. Hence sample 2 and 4 passed in aromatic test. We then verified our samples by our project guide, other faculties and classmates. They all told the same result. Hence in this way, we concluded that only sample 2 and sample 4 had aroma like actual beer.

CO_2 estimation by Gas Chromatography

PEAK REPORT (RAN)

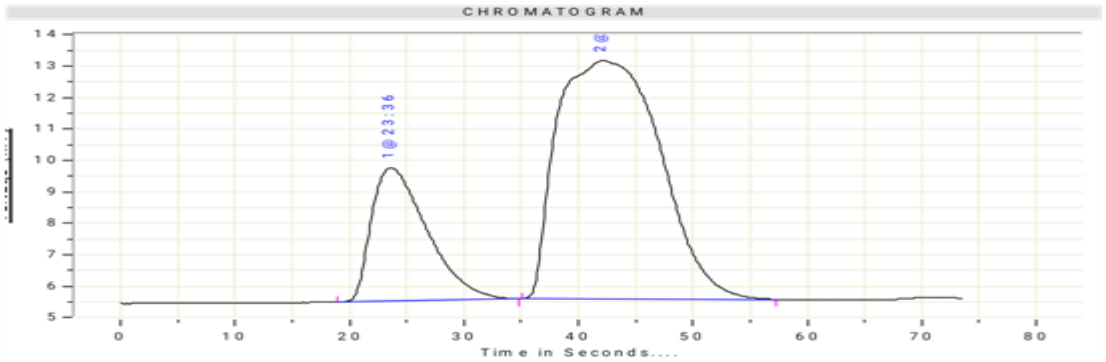

Table 1: Output of Gas - Chromatography

Peak No.	RT (Min)	Area mV-Sec	Height mV	RF	Amount (ML)	Amount%	Component Name
1	23:43	23.845	4.232	1.000	23.892	23.2931	Nitrogen
2	42:46	79.374	7.586	1.000	79.577	76.7717	Carbon di oxide

Alcohol concentration estimation using hydrometer

Table 2: Alcohol estimation in beer sample

S. No.	Sample	Intial pH of wort	Initial Specific Gravity of Wort	Final Specific gravity of beer	% alcohol={(Intial – Final)sp. Gravity}*(131.25)
1.	Sample 1	2.0	1.140	Nearly same as initial sp. gravity	Negligible
2.	Sample 2 (Control)	3.5	1.140	1.060	10.500
3.	Sample 3 (Honey)	3.5	1.140	1.080	7.875
4.	Sample 4	5.0	1.140	1.100	5.250

Glucose estimation by DNS method using spectrophotometer

Table 3:

S. No.	Standard sample of glucose (gm/100 mL)	Absorbance at 540 nm
1.	Sample 1 (10 gm)	0.237
2.	Sample 2 (20 gm)	0.422
3.	Sample 3 (30 gm)	0.762
4.	Sample (40 gm)	0.973

Absorbance of Beer sample

S. No.	Samples	Initial glucose conc. in g/L (Before Fermentation)	Absorbance of samples after fermentation at 540 nm	Final glucose conc. in g/L (After Fermentation)
1.	Sample 1	23.88	0.527	22.58
2.	Sample 2	23.88	0.126	5.875
3.	Sample 3	29.71	0.357	15.292
4.	Sample 4	23.88	0.337	14.667

Table 4: Estimation of glucose using absorbance

Microbiological testing of beer

Only sample 2, 3 and 4 were chosen for this test. Sample 1 had no fermentation, hence could not be analyzed. By observing the plates of all samples, we found that only sample 2 had a few colonies which were in standard limit. Rest two samples (Sample 3 & Sample 4) had a large number of colonies exceed from standard limit. Hence both the samples (Sample 3 & Sample 4) were failed in this taste and need to be pasteurized by using flash tunnel pasteurization equipment.

Conclusion

The present study demonstrates the feasibility of utilizing sorghum as an alternative substrate to barley for beer production. The fermentation process using *Saccharomyces cerevisiae* was successfully optimized, and both qualitative and quantitative analyses confirmed the suitability of sorghum-based formulations for brewing. The data obtained identified an optimum pH range that supported maximal production efficiency, highlighting the process parameters necessary for scalable application. Beyond technical validation, the results emphasize the potential of sorghum valorization in the brewing sector, offering a dual advantage of supporting farmers with an additional market avenue while providing the industry with a cost-effective and sustainable raw material. The findings thus contribute valuable insights into diversifying brewing substrates, advancing both agricultural and industrial sustainability.

Furthermore, the successful standardization of upstream and downstream processes underscores the reliability of sorghum as a consistent raw material for fermentation. The ability of sorghum-based wort to meet both physicochemical and sensory benchmarks demonstrates its competitiveness with traditional barley-based beer. This research also highlights the role of alternative grains in addressing the challenges of resource constraints and import dependency often faced by brewing industries in developing countries. The outcomes have direct implications for the valorization of underutilized crops, thereby promoting crop diversification and enhancing farmer resilience in fluctuating markets.

Future investigations should focus on scaling up the production process, conducting comprehensive sensory evaluations with consumer panels, and performing comparative cost–benefit analyses against conventional barley brewing. Additionally, genetic and agronomic improvement of sorghum varieties specifically tailored for brewing applications could further enhance yield and quality. Long-term studies on flavor stability, shelf-life, and commercial viability will be critical for industrial adoption. Collectively, this study establishes a scientific basis for integrating sorghum into modern brewing practices while simultaneously fostering agricultural sustainability and economic growth.

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