



Optimization of Initial Sugar Concentration for Fermented Malt Beverage Production from Thai Riceberry Rice Using Mixed-Culture Fermentation

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Abstract

The objective of this study was to evaluate the effects of different initial sugar concentrations on the fermentation behavior, chemical properties, and sensory quality of a fermented malt beverage produced from Thai Riceberry rice. Riceberry paddy was malted through soaking, germination in the dark, drying, and milling, after which the malt was mashed under a controlled temperature program to obtain wort at 10° Brix. The wort was then diluted to 2, 4, 6, 8, and 10° Brix and fermented for 15 days at room temperature using a mixed - starter culture of *Dekkera bruxellensis* and *Komagataeibacter intermedius* (1:1, 2% v/v). Samples were collected every three days to monitor total soluble solids (°Brix), alcohol content, and total acetic acid, and the final products were evaluated for consumer acceptance using a 9-point hedonic scale by 10 untrained panelists. Total soluble solids decreased sharply during the first six days across all treatments, indicating active substrate utilization. Fermentations initiated at 4–6°Brix showed the most efficient reduction in °Brix, with less than 50% of the initial value remaining after 15 days, whereas 8–10°Brix treatments retained more than half of their initial solids, suggesting incomplete fermentation. High-sugar treatments produced higher ethanol levels during the early stage, followed by a decline associated with its conversion to acetic acid, which increased steadily and reached a plateau, with the greatest acetic acid formation observed at 10°Brix. Sensory results showed that beverages produced at 4°Brix received aroma, taste, and overall liking scores comparable to the 8–10°Brix samples. Overall, an initial sugar concentration of 4–6°Brix was identified as optimal for achieving efficient fermentation, balanced metabolite profiles, and acceptable sensory quality, supporting the development of a low-sugar, Riceberry - based fermented malt beverage with commercial potential. The 4°Brix treatment yielded 4.5% alcohol and 1.6% w/v acetic acid, with an overall sensory score of 7.0.

Keywords: Riceberry rice, fermented malt beverage, *Dekkera bruxellensis*, *Komagataeibacter intermedius*, sugar concentration, acetic acid, sensory evaluation

1. Introduction

Thailand is recognized as one of the world's leading rice exporters, generating approximately USD 4.4 billion in annual export value and playing a central role in national food security and agricultural sustainability (Office of Agricultural Economics, 2021). However, the COVID-19 pandemic exposed the vulnerabilities of traditional agricultural markets, underscoring the need for innovation and diversification in rice-based value chains (Food and Agriculture Organization [FAO], 2020). In response, growing emphasis has been placed on transforming rice into higher-value, functional food and beverage products that offer enhanced nutritional, sensory, and commercial appeal (Champagne, 2008; Champrasert et al., 2020).

Among premium Thai rice varieties, Riceberry rice has attracted considerable interest due to its distinctive dark-purple pigmentation and exceptional nutritional composition. It is rich in anthocyanins, phenolic compounds, antioxidants, vitamins, and key minerals including zinc, iron, and selenium (Sompong et al., 2011; Sutharut & Sudarat, 2012). This nutritional attribute has been associated with various health benefits, including reduced cardiovascular risk, improved glycemic regulation, and anti-inflammatory properties. Germinated Riceberry brown rice exhibits even greater functional potential, as germination activates endogenous enzymes, resulting in increased levels of γ -aminobutyric acid (GABA), free amino acids, antioxidant compounds, and more digestible carbohydrates (Moongngarm & Saetung, 2010). These biochemical enhancements also impart a naturally sweeter and smoother sensory profile, making germinated Riceberry an ideal substrate for developing functional beverages aimed at health-conscious consumers (Patil & Khan, 2011).



Parallel to these developments, fermented malt beverages (FMBs) have gained global prominence as a category encompassing health-oriented, low-alcohol, and naturally fermented drinks (Nummer, 2013). Traditional examples include kombucha, kvass, and fermented cereal-based beverages, which have demonstrated probiotic potential, bioactive compound formation, and improved digestibility (Dimidi et al., 2019; Jayabalan et al., 2014). Developing Riceberry-based FMBs aligns strategically with contemporary consumer preferences for functional beverages and provides an effective pathway for enhancing the economic value of Thai specialty rice (Granato et al., 2010). A critical process parameter in FMB production is the initial sugar concentration of the wort, which directly influences fermentation kinetics, metabolite formation patterns, microbial ecology, product stability, caloric density, and overall sensory quality (Qiao et al., 2020). Lower sugar formulations offer multiple advantages, including reduced caloric content, minimized residual fermentable substrates that could trigger undesirable post-packaging fermentation, decreased production costs, and alignment with public health recommendations for sugar reduction (Te Morenga et al., 2013; World Health Organization [WHO], 2015). However, the initial sugar concentration fundamentally modulates microbial metabolic activity and fermentation completeness, necessitating systematic empirical evaluation to determine optimal conditions for specific substrate microorganism combinations (Marsh et al., 2014).

Microbial culture selection is equally critical in defining the chemical and sensory attributes of fermented beverages. *Dekkera bruxellensis*, a non-conventional yeast, is valued for its ability to efficiently utilize sugars effectively even at low concentrations while producing complex aroma compounds that contribute depth to fermented products (Smith & Divol, 2016). *Komagataeibacter intermedius*, an acetic acid bacterium, contributes organic acids and bioactive polysaccharides that enhance acidity, provide potential functional benefits, and influence mouthfeel (Coton et al., 2017). Co-fermentation with these two microorganisms offers the potential to achieve a beverage with balanced acidity, moderated sweetness, and distinctive aromatic complexity (Jayabalan et al., 2014).

Given the nutritional advantages of Riceberry rice, the increasing consumer demand for low-sugar functional beverages, and the synergistic potential of mixed-culture fermentation systems, a systematic investigation of Riceberry-derived fermented malt beverages represents both a timely research priority and a valuable contribution to agricultural innovation. Despite growing interest in cereal-based fermented beverages and the health benefits associated with pigmented rice varieties, comprehensive studies examining the interactive effects of substrate composition (particularly Riceberry malt), initial sugar concentration, and mixed-culture fermentation dynamics remain limited in the scientific literature

Therefore, this study systematically investigates the production and fermentation behavior of Riceberry malt beverages at varying initial sugar concentrations (2, 4, 6, 8, and 10°Brix), with the objectives of optimizing fermentation efficiency, characterizing metabolite production profiles, and evaluate sensory acceptability. The findings are anticipated to provide evidence-based recommendations for developing a nutritionally enhanced, organoleptically balanced, low-sugar fermented beverage with translational potential for commercial applications, while simultaneously contributing to the value-added utilization of Thai specialty rice varieties and supporting agricultural diversification initiatives.

2. Objectives

This study aimed to investigate the effects of different initial sugar concentrations on the fermentation performance and product quality of a Riceberry-based fermented malt beverage. The specific objectives were to:

1. Evaluate changes in total soluble solids (°Brix), alcohol content, and acetic acid production over 15 days of fermentation under varying initial sugar levels.
2. Examine the metabolic interactions between *Dekkera bruxellensis* and *Komagataeibacter intermedius* across varying sugar concentrations.
3. Identify the sugar level that promotes efficient fermentation with minimal residual solids.
4. Assess the sensory acceptance of the final beverages to determine if low-sugar formulations are comparable to higher-sugar products.
5. Recommend the optimal initial sugar concentration suitable for cost-effective and commercially viable Riceberry fermented beverage production.



3. Materials and Methods

3.1 Materials & Equipment

Riceberry paddy rice harvested in 2021 was sourced from the Nong Sa Rai Community Enterprise Group, Phanom Thuan District, Kanchanaburi Province, Thailand, and only grains that had passed the dormancy period were used for malt preparation. Pure cultures of *Dekkera bruxellensis* (yeast) and *Komagataeibacter intermedius* (acetic acid bacterium) were obtained from the Thailand Institute of Scientific and Technological Research (TISTR) and maintained under appropriate laboratory conditions prior to inoculation.

Malt preparation and wort extraction were carried out using a moisture analyzer (Sartorius, Germany) and a laboratory mill for fine grinding. Wort production employed a temperature-controlled mashing system (Brewzella, Australia) and a digital Brix refractometer (AMR 100, USA) for soluble solids measurement. Fermentation trials were conducted in sterilized glass jars, with sample preparation supported by an autoclave (Sanyo, Netherlands) and a shaker incubator (Gerhardt, Germany). Alcohol content was determined using an ebulliometer (Dujardin, France), while pH and acetic acid measurements were performed using a pH meter (Sartorius, Germany) and a standard titration apparatus. Product quality evaluation used a visible spectrophotometer (Genesys 20, Thermo Spectronic, USA).

All chemicals used for analytical procedures, including Folin-Ciocalteu reagent, sodium carbonate, phenol reagent, sodium hypochlorite, borate reagents, DPPH, methanol, absolute ethanol, sodium hydroxide, phenolphthalein, D-saccharic acid-1,4-lactone, and glucose standards, were analytical grade and used as received without further purification.

3.2 Preparation of Riceberry Malt

Riceberry paddy was cleaned and subjected to an initial seed selection process. The grains were soaked in water at a ratio of 1:3 (paddy rice:water, w/v) for 5 minutes to separate floating debris. Only the sunken seeds were used for malting.

The selected grains were then soaked at room temperature with intermittent aeration every 12 hours for 1 hour. After soaking, excess water was drained, and the grains were spread to allow adequate aeration at ambient temperature (Figure 1).



Figure 1 Riceberry paddy soaking process. Illustration of the initial soaking step used to prepare Riceberry paddy prior to germination.

Once the grains reached a stable moisture content, they were germinated in darkness for 60 hours at room temperature and approximately 90% relative humidity. Germination trays were lined with water-moistened tissue paper, and the grains were evenly distributed on the surface. The trays were placed inside zip-lock bags and further enclosed in black plastic bags to prevent light exposure. Moisture was maintained by spraying water every 6 hours to ensure the relative humidity remained above 90% (Figure 2).

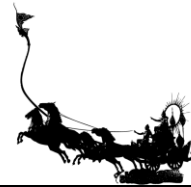


Figure 2 Germination procedure for Riceberry paddy.

Germination was conducted in dark, high-humidity conditions to activate endogenous enzymes for malt production.

3.3 Wort Extraction

The germinated malt was dried using a tray dryer at 55 °C for 24 hours and then ground into a fine powder. Wort extraction was performed by mixing the malt powder with water at a ratio of 1:5 (w/v). The mixture was filtered to remove solids, and the liquid fraction was subjected to a multi-step temperature-controlled mashing program designed to optimize starch gelatinization and enzymatic saccharification:

- Protein rest: 52°C for 30 minutes (protease activity; peptide formation)
- β -amylase saccharification: 63°C for 60 minutes (maltose production)
- α -amylase liquefaction: 78°C for 60 minutes (dextrin hydrolysis)
- Mash-out: 79°C for 5 minutes (enzyme inactivation)

This temperature program facilitates sequential activation of endogenous hydrolytic enzymes (proteases, β -amylase, α -amylase) while progressively denaturing enzymes to prevent excessive hydrolysis (Mundy & Varjú, 2005). Following mashing, the wort was cooled rapidly to 20°C to minimize non-enzymatic browning and microbial contamination, filtered through diatomaceous earth (Celite 545; particle size 10–40 μ m) to remove insoluble particulates and spent grain residues, and sterilized by autoclaving at 121°C for 15 minutes (Marsh et al., 2014). The sterile wort exhibited an initial total soluble solids content of 10°Brix, as measured by digital refractometry.

3.4 Fermentation Procedure

The sterilized Riceberry wort with an initial 10°Brix was diluted using distilled water to obtain experimental treatments of 10, 8, 6, 4, and 2°Brix. Each treatment was distributed into 250-mL fermentation jars, with three replicates per treatment (Figure 3).

Fermentation Procedure

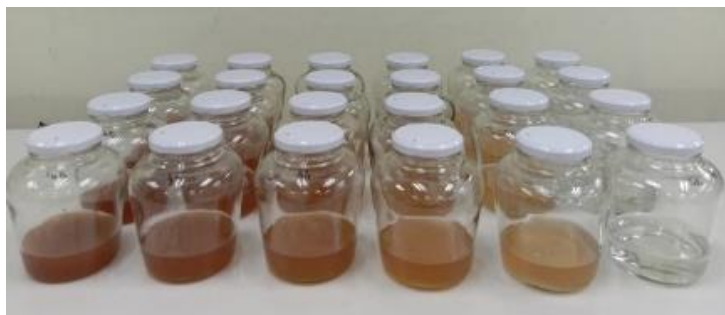
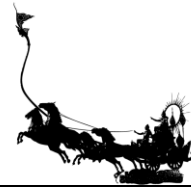


Figure 3 Fermentation setup for Riceberry malt beverages.

Mixed-culture fermentation using *Dekkera bruxellensis* and *Komagataeibacter intermedius* inoculated into Riceberry wort diluted to target Brix levels.



The inoculum of *Dekkera bruxellensis* was prepared by transferring one loopful of culture into YM broth and incubating it on a rotary shaker at 190 rpm at room temperature for 24 hours. The resulting culture was enumerated, and the cell suspension was diluted with 0.1% peptone to obtain an initial concentration of 1.5×10^8 cells/mL. Similarly, the inoculum of *Komagataeibacter intermedius* was generated by inoculating one loopful of culture into GYE broth and shaking at 190 rpm at room temperature for 48 hours. The cells were then counted and diluted with 0.1% peptone to achieve an initial concentration of 1.5×10^8 cells/mL.

A mixed microbial suspension containing *Dekkera bruxellensis* and *Komagataeibacter intermedius* at a 1:1 volume ratio was prepared. Each fermentation jar was inoculated with 2% (v/v) mixed starter culture. Fermentation vessels were sealed with sterile cotton plugs to permit gas exchange while preventing contamination and were incubated at ambient temperature ($25 \pm 2^\circ\text{C}$) for 15 days without agitation. Samples were collected every 3 days for physicochemical analysis. The following parameters were measured.

Total soluble solids ($^\circ\text{Brix}$) were measured directly using a digital refractometer at 20°C , as described in AOAC Method 932.12 (AOAC International, 2019).

Ethanol content (% v/v) was determined using a modified ebulliometer method, in which ethanol lowers the boiling point of the sample proportionally to its concentration (Iland et al., 2004). The method involves heating a fixed volume of sample to boiling and measuring the vapor temperature, which is correlated to ethanol concentration via calibration curves.

Total acetic acid (%w/v) was quantified by acid-base titration according to AOAC Method 942.15 (AOAC International, 2019). A known volume of sample was titrated against standardized 0.1 N sodium hydroxide using phenolphthalein as an indicator, and results were expressed as grams of acetic acid per liter.

Upon completion of fermentation (day 15), beverages were subjected to pasteurization at 75°C for 15 minutes to inactivate viable microorganisms and stabilize the products.

Sensory Evaluation

Consumer acceptance was assessed using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely), following established guidelines for affective testing. A panel of 10 untrained consumers (5 males, 5 females; age range 22–35 years) evaluated pasteurized beverages for aroma, taste, and overall acceptability. Although standard practice recommends panels of 50–100 consumers for commercial product development, preliminary screening with smaller panels (10–30 participants) is acceptable for research purposes and initial product optimization (Stone & Sidel, 2004). Samples (approximately 30 mL) were served in transparent plastic cups coded with random three-digit numbers and presented in randomized order under ambient lighting and temperature conditions. Panelists were provided with water and unsalted crackers for palate cleansing between samples.

3.5 Statistical analysis

A completely randomized design (CRD) was used to study the effects of sugar concentration (0–10%) on fermentation quality parameters, including total soluble solids, alcohol content, and acetic acid concentration. For sensory evaluation, a randomized complete block design (RCBD) was employed, with panelists serving as blocks. Mean values were compared using Duncan's New Multiple Range Test (DMRT) at a significance level of 0.05 via SPSS version 12.0.

4. Results and Discussion

4.1 Changes in Total Soluble Solids During Fermentation

Figure 4 shows that total soluble solids ($^\circ\text{Brix}$) decreased substantially across all treatments during the initial six days of fermentation, indicating rapid microbial utilization of fermentable carbohydrates including glucose, maltose, and maltotriose. This pattern is consistent with established fermentation kinetics wherein exponential microbial growth coincides with maximal substrate consumption rates (Coton et al., 2017; Villarreal-Soto et al., 2018). The initial $^\circ\text{Brix}$ reduction reflects primarily the metabolic activity of *D. bruxellensis*, which preferentially ferments simple sugars during the early fermentation phase when oxygen availability supports both respiratory and fermentative metabolism (Smith & Divol, 2016).

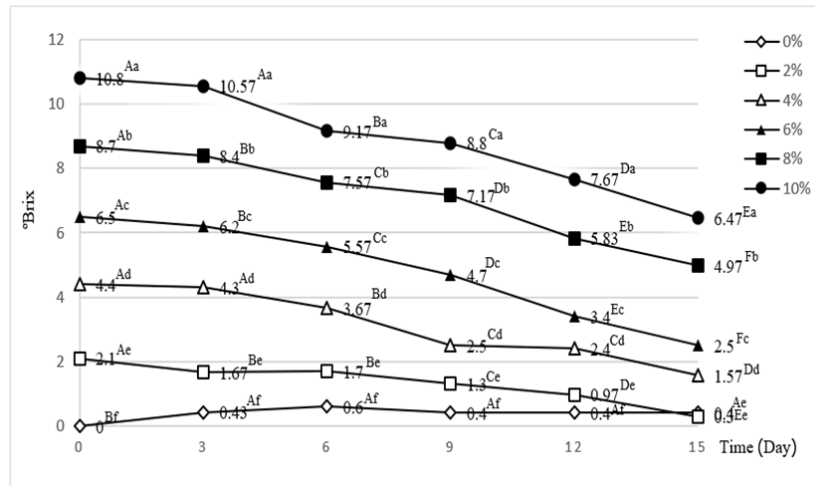
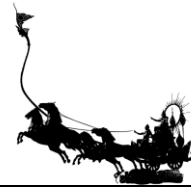


Figure 4 Changes in total soluble solids ($^{\circ}$ Brix) during 15 days of fermentation at different initial sugar concentrations. Values represent mean with different uppercase letters indicate significant differences across sampling days ($p < 0.05$), and lowercase letters indicate differences among initial $^{\circ}$ Brix treatments ($p < 0.05$).

Higher initial sugar concentrations (8–10 $^{\circ}$ Brix) exhibited accelerated early-stage $^{\circ}$ Brix reduction; however, substantial residual soluble solids (>50% of initial values) persisted after 15 days, suggesting incomplete fermentation. This phenomenon may be attributed to several factors: (1) accumulation of inhibitory metabolites including ethanol and organic acids that suppress microbial activity at elevated concentrations (Coton et al., 2017); (2) progressive nutrient depletion, particularly assimilable nitrogen sources essential for yeast growth (Bisson, 1999); and (3) preferential utilization of monosaccharides and disaccharides, leaving less-fermentable oligosaccharides in solution (Bamforth, 2006).

In contrast, treatments initiated at 4–6 $^{\circ}$ Brix demonstrated continuous $^{\circ}$ Brix reduction throughout the 15-day fermentation period, culminating in residual soluble solids representing less than 50% of the initial concentrations. This indicates more efficient substrate conversion under moderate initial sugar conditions, where microbial metabolism is sustained without inhibitory metabolite accumulation reaching critical thresholds (Jayabalan et al., 2014). The 2 $^{\circ}$ Brix treatment exhibited minimal absolute $^{\circ}$ Brix change due to limited substrate availability, resulting in constrained microbial growth and metabolite formation.

These findings align with previous investigations of kombucha and related fermented beverages demonstrating that moderate initial sugar concentrations (4–6% w/v, approximately 4–6 $^{\circ}$ Brix) optimize fermentation efficiency by balancing substrate availability against inhibitory metabolite accumulation (Qiao et al., 2020; Yupakanit & Soithongsuk, 2020). Notably, the study by Villarreal-Soto et al. (2018) reported that kombucha fermentations with 2–5% initial sugar achieved more complete substrate utilization within 14 days compared to 8–10% sugar treatments, supporting the present observations with Riceberry wort.

4.2 Alcohol Production Dynamics

Changes in alcohol production versus fermentation time is shown in Figure 5.

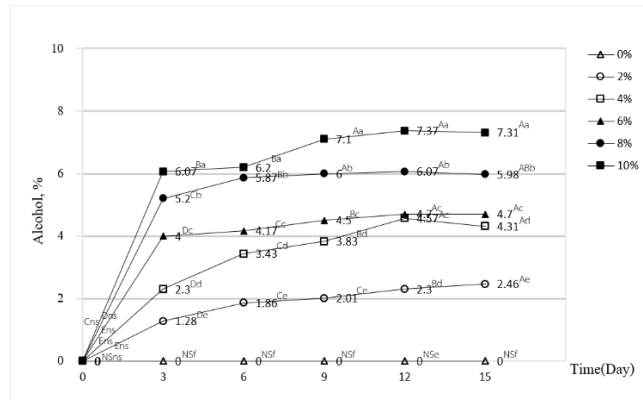


Figure 5 Alcohol production during 15 days of fermentation at different initial concentrations.

Values represent mean with different uppercase letters indicate significant differences across sampling days ($p < 0.05$), and lowercase letters indicate differences among initial °Brix treatments ($p < 0.05$).

Alcohol production followed patterns expected from sugar consumption. In high-sugar treatments (8–10%), ethanol concentration increased rapidly within the first three days, reflecting the high availability of fermentable substrates for *Dekkera bruxellensis*. However, after day 3, ethanol levels declined progressively. This reduction can be attributed to its conversion into acetic acid by acetic acid bacteria, which utilize ethanol as a primary substrate for organic acid synthesis (Smith & Divol, 2016). Acetic acid bacteria, including *Komagataeibacter intermedius*, are obligate aerobes that preferentially utilize ethanol as a carbon and energy source when oxygen is available, converting it quantitatively to acetic acid via the incomplete oxidation pathway (Gullo et al., 2014).

Conversely, low-sugar treatments (2–6%) exhibited slower ethanol production due to limited substrate availability. In the 0% sugar treatment, ethanol did not accumulate, as no fermentable carbon source was present. The ethanol-to-acid conversion pattern observed aligns closely with previous reports in kombucha fermentation systems, where ethanol peaks early and then declines as acetic acid bacteria become dominant around days 3–10 (Chakravorty et al., 2016). This confirms that the mixed-culture fermentation using *Dekkera* and *Komagataeibacter* follows conventional metabolic shifts seen in symbiotic fermentations.

4.3 Acetic Acid Formation During Fermentation

Acetic acid accumulation during 15 days of fermentation at different initial sugar concentrations is shown in Figure 6.

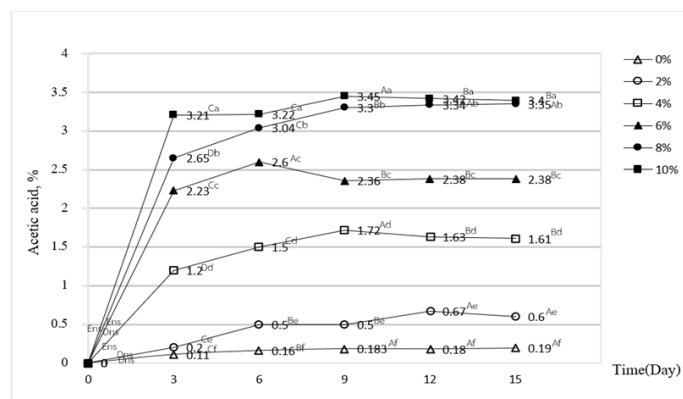


Figure 6 Acetic acid accumulation during 15 days of fermentation at different initial sugar concentrations.

Values represent mean with different uppercase letters indicate significant differences across sampling days ($p < 0.05$), and lowercase letters indicate differences among initial °Brix treatments ($p < 0.05$).



Acetic acid concentration increased steadily across all treatments until reaching a maximum, after which levels plateaued. This pattern reflects the metabolic role of acetic acid bacteria, which convert ethanol into acetic acid during mid-fermentation. The highest acetic acid production occurred in the 10% sugar treatment, reaching its peak around day 9, likely because of greater ethanol availability during the early stage.

As fermentation progressed, accumulation of acetic acid and associated decreases in pH may have inhibited microbial activity, leading to the observed stabilization (Qiao et al., 2020). This is consistent with existing literature indicating that acetic acid levels of 3–5% w/v or pH values below approximately 2.5 suppress acetic acid bacteria metabolism (Gullo et al., 2014).

4.4 Sensory Evaluation of Fermented Riceberry Beverage

Sensory assessment (9-point hedonic scale) conducted after 15 days is summarized in Table 1.

Table 1 Sensory evaluation scores of Riceberry fermented malt beverages after 15 days of fermentation.

Scores represent mean \pm standard deviation based on a 9-point hedonic scale.

Attributes	Score					
	0%	2%	4%	6%	8%	10%
Aroma	1.30 \pm 0.00 ^C	5.65 \pm 0.15 ^B	6.85 \pm 0.10 ^A	6.88 \pm 0.15 ^A	6.89 \pm 0.21 ^A	6.93 \pm 0.21 ^A
Taste	1.30 \pm 0.00 ^C	5.46 \pm 0.04 ^B	6.98 \pm 0.16 ^A	7.02 \pm 0.20 ^A	7.07 \pm 0.24 ^A	7.11 \pm 0.01 ^A
Overall liking	1.30 \pm 0.00 ^C	5.66 \pm 0.01 ^B	6.83 \pm 0.17 ^A	6.94 \pm 0.21 ^A	6.80 \pm 0.16 ^A	6.85 \pm 0.11 ^A

Note. Values are presented as mean \pm standard deviation (n = 10). Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Sensory assessment (9-point hedonic scale) conducted after 15 days of fermentation revealed that beverages produced at 4% initial sugar achieved moderate acceptance in aroma, taste, and overall liking (6.65–7.03). Importantly, these scores were not significantly different ($p < .05$) from beverages fermented with 8–10% sugar.

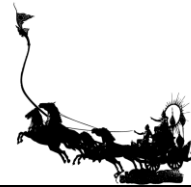
Panelists described the products as having a dark brown color, characteristic aroma, and naturally sour taste typical of fermented beverages. Samples fermented with 0–2% sugar were rated significantly lower, reflecting insufficient flavor development due to limited metabolite formation.

The finding that 4% sugar yields sensory quality comparable to higher-sugar formulations is noteworthy because it indicates potential cost savings and reduces risks associated with residual sugar, such as secondary fermentation. This outcome supports earlier studies that low-sugar kombucha formulations (2–6%) maintain acceptable sensory profiles while improving product stability (Yupakanit & Sroitongsuk, 2020), which traditionally have been considered necessary to achieve acceptable sensory quality in fermented beverages (Qiao et al., 2020).

Taken together, the results demonstrate that moderate initial sugar levels (4–6%) most effectively balance fermentation efficiency, metabolite production, and sensory acceptability. High sugar levels (8–10%) enhanced early alcohol production but left excess unfermented sugar and contributed to unstable ethanol dynamics due to conversion into acetic acid. Low sugar (0–2%) led to incomplete flavor development. Comparisons with previous kombucha literature indicate that Riceberry malt behaves similarly to traditional tea substrates in sugar utilization, ethanol-acid dynamics, and microbial succession. This highlights Riceberry's viability as a functional raw material for fermented beverages.

5. Conclusion

This study demonstrated that initial sugar concentration plays a critical role in shaping the fermentation kinetics, metabolite production, and sensory quality of Riceberry-based fermented beverages produced using *Dekkera bruxellensis* and *Komagataeibacter intermedius*. Across all treatments, total soluble solids ($^{\circ}$ Brix) decreased markedly during the early stages of fermentation, with the 4–6% sugar systems showed the most efficient reduction and minimal residual substrates by day 15. Higher initial sugar levels (8–10%) promoted rapid early fermentation but resulted in substantial residual $^{\circ}$ Brix, indicative of incomplete sugar utilization.



Alcohol production patterns further revealed the metabolic interaction between yeast and acetic acid bacteria. High-sugar treatments generated higher ethanol concentrations initially, followed by a decline as ethanol was metabolized into acetic acid. Correspondingly, acetic acid levels increased steadily and reached a plateau once microbial activity was inhibited by accumulating organic acids. These metabolic trends are consistent with reported behavior in kombucha-type fermentations, supporting the suitability of Riceberry malt as an alternative substrate.

Sensory evaluation highlighted that beverages produced with 4% initial sugar received liking scores comparable to those produced with higher sugar levels (8–10%). This indicates that acceptable flavor, aroma, and overall quality can be achieved with significantly lower sugar input. From a production standpoint, using 4–6% initial sugar offers distinct advantages: reduced raw material costs, lower residual sugar that minimizes risks of secondary fermentation in the final product, and improved fermentation efficiency.

Several limitations should be acknowledged in this study. Beyond the sensory panel size limitation already mentioned, we recognize that our study was conducted at a laboratory scale under controlled conditions. Scale-up to pilot or industrial production may present additional challenges in maintaining consistent fermentation conditions and product quality. Additionally, the study focused on a specific temperature range ($25 \pm 2^\circ\text{C}$) and fermentation time (15 days), which represents typical ambient conditions in Thailand but may not reflect optimal conditions for all production scenarios.

Overall, the findings suggest that an initial sugar concentration of 4–6% is optimal for producing a stable, palatable, and economically viable Riceberry fermented beverage. Future studies should expand sensory panels to at least 30 participants, as recommended in standard sensory evaluation guidelines, and explore shelf-life stability and microbial dynamics to support commercial-scale development.

6. Acknowledgements

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