# Effect of Low Sugar Content on Kombucha from RD 43 Rice Fermentation by Starter Culture of Dekkera and Komagataeibacter

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**Abstract**

This research aimed to investigate the optimal sugar concentration for commercial low sugar kombucha production from RD 43 rice. The fermentation qualities and sensory attributes of kombucha using different concentrations of sugar (0, 2, 4, 6, 8, and 10%) from RD 43 wort and pure starter culture of *Dekkera bruxellensis* and *Komagataeibacter intermedius* were evaluated. The fermentation was carried out by preparing an initial cell suspension of $1.5\times10^{8}$ cell/ml with a ratio of *Dekkera bruxellensis*: *Komagataeibacter intermedius* as 1:1 for 2% of inoculum size. The fermentation condition was a static culture at ambient temperature for 15 days and samples were taken every 3 days until 15 days for fermentation quality analysis. The results showed that using a low starting sugar concentration of 2-6% could reduce the residual sugar in the final product. The chemical quality of kombucha differed according to the initial sugar concentration. The sensory test indicated that RD 43 kombucha from 4% initial sugar had the score in the level of moderately like. The obtained results from this study should be beneficial for entrepreneurs not only to reduce costs and help to prevent the secondary fermentation in the bottle but also to increase the value of rice. The low sugar kombucha from RD 43 rice could be commercialized in the future.

**Keywords:** *RD 43, Rice kombucha, low percent of sugar, Dekkera, Komagataeibacter*

## 1. Introduction

Kombucha is a very popular drink nowadays. It has a global market value of US$1.5 billion in 2018. It has a market growth of *23%* from 2014 to 2018 and is likely to continue growing. The global market value is expected to reach $3.5-5.0 billion in 2025 *(*Kim & Adhikari, *2020).* Kombucha is a living beverage filled with the activities of many enzymes. It has a slightly sour taste and refreshing carbonation after complete fermentation. The remaining alcohol content is a low-level amount of 0.5-1 percent, and the total sugar content is vary depending on the style of fermentation and raw materials (Ebersole et al., 2017). To ensure that the quality of the product will not change over its shelf life by secondary fermentation in the bottle, there are commercial low-sugar kombucha products sold in the United States. The common ingredients of kombucha are sugar and black tea. The process begins by adjusting the sugar concentration to 3-10%. Kombucha fermentation breaks down sucrose into glucose and fructose for conversion to alcohol and many organic acids. Besides organic acids, many types of intermediate substances are beneficial to the body such as glucuronic acid, oxalic acid, butyric acid, lactic acid, tartaric acid, malic acid, and citric acid including glucuronidase, which have activities associated with antimicrobial and antitoxic effects for chemotherapy (May et al., 2019; Srinivasan et al., 1997). Kombucha also contains D-saccharic acid 1,4 lactone (DSL), a substance that promotes the liver's detoxification and carcinogenicity (Martinez et al., 2018.; Zhiwei et al., 2010). Consuming kombucha can lower blood cholesterol levels *(*Aleei et al., 2020). It also helps strengthen the inhibition of alpha-amylase enzyme activity in the plasma and pancreas resulting in an increase in insulin levels and glucose tolerance as well as a suppression of blood sugar levels (Aloulou et al. 2012). A survey of Thai people's health by the Public Health Systems Research Institute between 2009 and 2014 found that Thai people had more diabetes at any age, from 6.9 percent to 8.9 percent. The concerning groups are adolescence and working age (Aekplakorn et al., 2011*).* According to sugar content in kombucha, Ebersole (2016) reported that kombucha products in general retail five of the eight manufacturers in the United States contain sugar of more than 20 percent, which are different from the average stated on the product label. Moreover, many commercial products contain an alcohol content of 4-11 percent (by volume) above the requirement of no higher than 0.5% non-alcohol beverages (United States Federal law), which is unhealthy for consumers especially children and pregnant women (Chan et al., 2021; Ebersole et al., 2017). Malbasa et al. (2008a) concluded that 7% sucrose content was the optimal concentration for kombucha fermentation.

The study on the effect of *Dekkera and Komagataeibacter* ratio on the quality of kombucha with different sugar content was reported in 2020 (Puangwerakul and Soitongsuk, 2020). It was found that when using a ratio of 1:1, both microorganisms showed good growth and fermentation activity at all sugar concentrations (10, 12, 14, 16, 18, and 20 percent), but the fermentation did not end in a period of 6 days. The kombucha quality on day 6 had an alcohol content of 2.25-4.83% and acetic acid content of 2.46-3.87%. It was concluded that it is not necessary to use a sugar concentration higher than 10% because using 10% sugar can produce excess acetic acid and alcohol content including more remaining sugar in kombucha drinks. However, it is not clear how much sugar concentration is suitable for good kombucha fermentation and is accepted by consumers for commercial application. Therefore, this study aimed to answer these questions

## 2. Objectives

To evaluate the effect of different sugar concentrations on the fermentation of kombucha from RD 43 rice varieties and the organoleptic properties of the product

## 3. Materials and Methods

RD 43 paddy rice (harvested in November 2021) from Nong Sarai Farmer Community Enterprise Group Kanchanaburi was used to prepare rice malt as the method of the previous research (Mueanfan & Yupakanit, 2020). The paddy rice was stored for 4 weeks until passed the dormancy period. Before use, the paddy rice was soaked in water at 30 °C for 60 hours to obtain 95% germination. All chemical agents are analytical grade derived from Merck (Darmstadt, Germany).

### *3.1 Preparation of kombucha starter*

*Dekkera bruxellensis and Komagataeibacter intermedius* were prepared by the method described by Puangwerakul and Soitongsuk (2020*). D. bruxellensis* 1 loopful was cultured in YM liquid medium, it was shaken at 190 rpm at ambient temperature for 24 hr. The cells were then counted and diluted with 0.1% peptone solution to prepare an initial number of $1.5x10^8$ cells/ml. For the preparation of *K. intermedius*, 1 loopful was cultured in liquid GYE medium, it was shaken at 190 rpm at ambient temperature for 48 hr. The cells were then counted and diluted with 0.1% peptone solution to prepare an initial number of $1.5x10^8$ cells/ml.

The starter culture was composed of *D. bruxellensis* and *K. intermedius* at a ratio of 1:1. It was prepared in 2% inoculum size of 300 ml of the fermented flask by pipette of 3.0 ml of yeast cell solution and 3 ml of bacterial cell solution into the fermentation flask.

### *3.2 Wort preparation*

10 kilograms of RD 43 malt were mixed with 50 kilograms of water in a 65 L-mashing machine *(BrewZilla* 65L-Gen3.1.1, Australia). The temperature programs were performed as follows; 52 °C for 30 min, 63 °C for 60 min, 78 °C for 60 min and held at 79 °C for 5 min. Then, it was cooled to 20 °C, adjusted weight to 60 kilograms, and filtered through a filter press to obtain the clear wort, which had the quality following the EBC benchmark. The clear wort was then diluted to 8, 6, 4, 2, and 0 Brix with double distilled water.

*3.3 Fermentation*

The 300 mL of clear RD 43 wort with different sugar concentrations were filled in a 500 mL sterilized fermentation glass bottle, the lids were closed and the bottles were cooled down to ambient temperature. The starter of yeast and bacteria were added and the glass bottles were closed with sterilized cotton cheesecloth, then they were incubated at ambient temperature for 15 days.

*3.4 Quality analysis during fermentation*

Samples were taken on days 0, 3, 6, 9, 12, and 15 to measure quality parameters as followed: °Brix with a hand refractometer, alcohol content by Vinometer, total acidity as acetic acid was determined by titration method with 0.1 normal NaOH solution with phenolphthalein as an indicator, and thickness of the biofilm in millimeters by using a measuring tape.

Sensory evaluation of RD 43 kombucha samples was carried out by 10 trained tasters who had experience and were familiar with kombucha beverages. The 9 scale point –Hedonic was designed as 1-9 scores for evaluation on aroma, taste, and overall preferences by 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislikes slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = likes extremely.

***3.5 Statistical analysis***

All assays were performed in triplicate and the data were recorded as means ± standard deviation. The obtained data were analyzed by one-way ANOVA and tests of significance were carried out using Duncan's New Multiple Range Test (DMRT) with SPSS for windows version 12.0.

## 4. Results and Discussion

The effect of different sugar concentrations on °Brix, alcohol, and acetic acid content including bio-cellulose thickness are shown in Figures 1-4

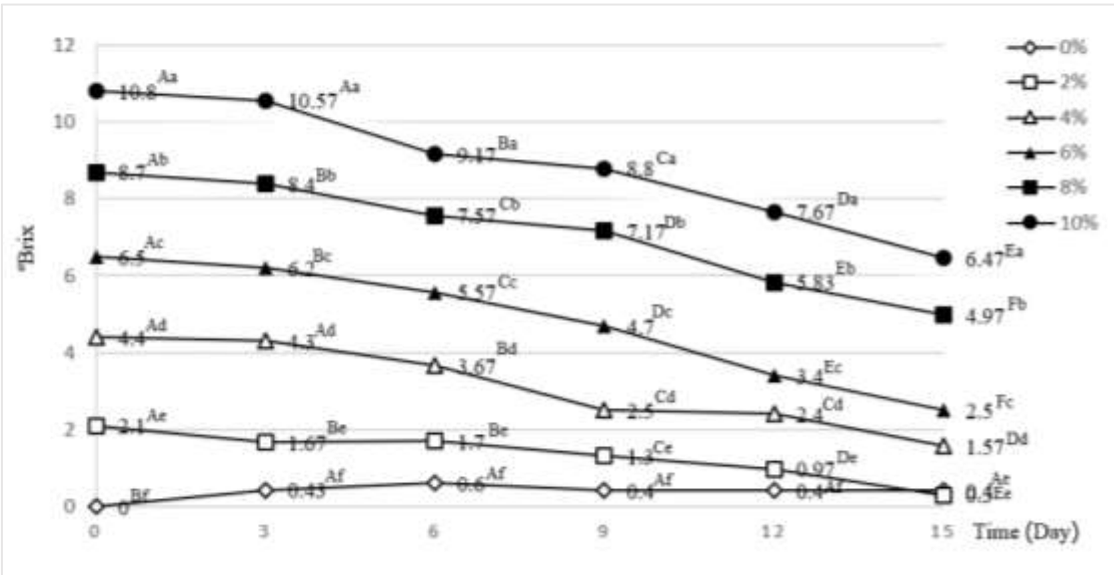


**Figure 1** Changes in °Brix throughout the fermentation period (15 days). Data with different superscripts A, B, C, D, E, and F represent a significant difference ($p \leq 0.05$) of °Brix of the same treatment (initial sugar concentration) during the fermentation period. Data with different superscripts a, b, c, d, e, and f represent a significant difference ($p \leq 0.05$) of °Brix of each treatment on the same fermentation day.

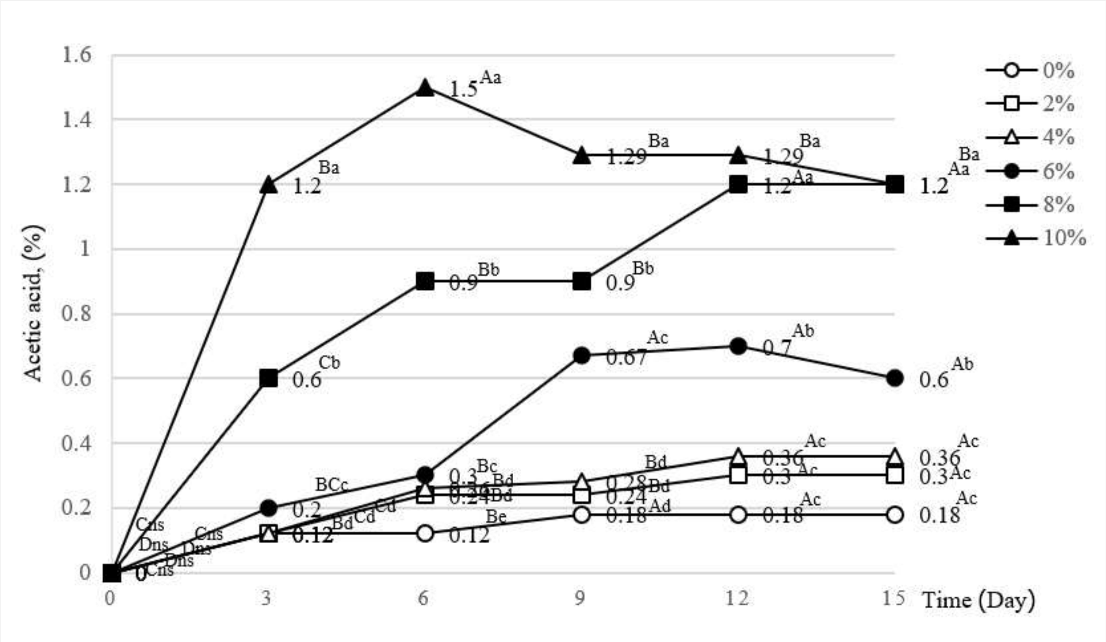


**Figure 2** Changes in acetic acid contents throughout the fermentation period (15 days). Data with different superscripts A, B, C, D, E, and F represent a significant difference ($p \leq 0.05$) in the acetic acid content of the same treatment (initial sugar concentration) during the fermentation period. Data with different superscripts a, b, c, d, e, and f represent a significant difference ($p \leq 0.05$) in the acetic acid content of each treatment on the same fermentation day.

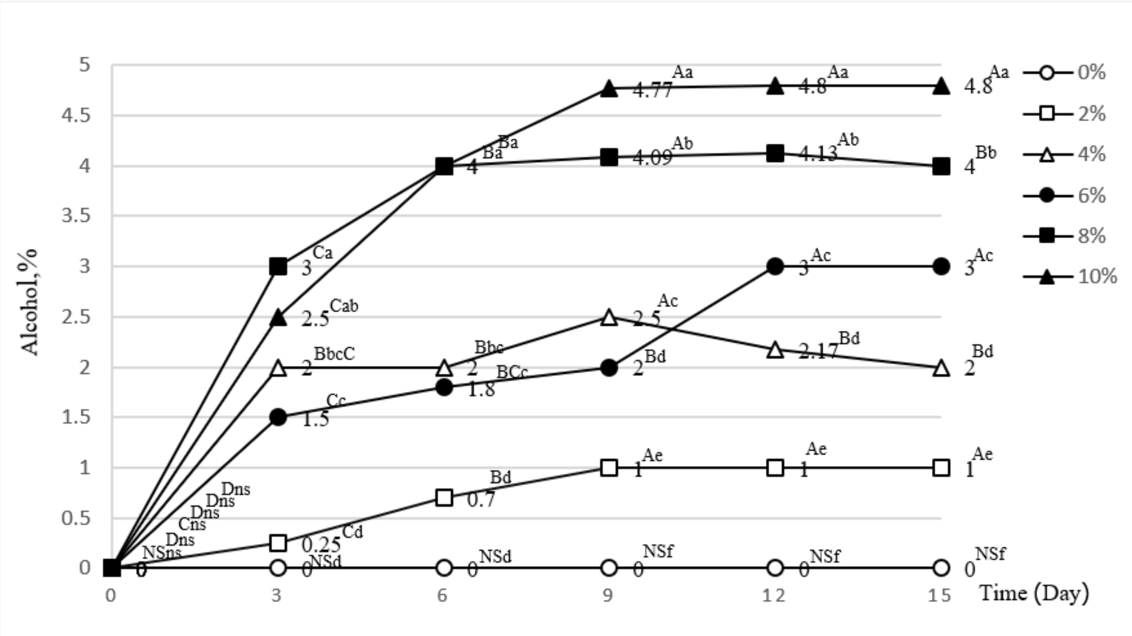


**Figure 3** Changes in alcohol content throughout the fermentation period (15 days). Data with different superscripts A, B, C, D, E, and F represent significant differences ($p \leq 0.05$) in alcohol content of the same treatment (initial sugar concentration) during the fermentation period. Data with different superscripts a, b, c, d, e, and f represent a significant difference ($p \leq 0.05$) in the alcohol content of each treatment on the same fermentation day.

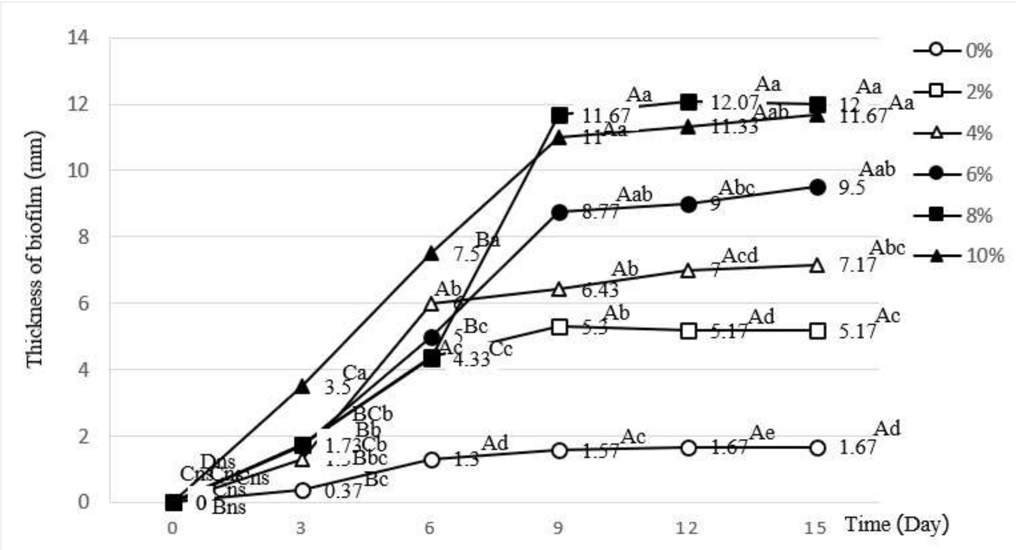


**Figure 4** Changes in biofilm thickness throughout the fermentation period (15 days). Data with different superscripts A, B, C, D, E, and F represent a significant difference ($p \leq 0.05$) in biofilm thickness of the same treatment (initial sugar concentration) during the fermentation period**.** Data with different superscripts a, b, c, d, e, and f represent a significant difference ($p \leq 0.05$) in biofilm thickness of each treatment on the same fermentation day.

It was found that during fermentation sugar content decreased while alcohol content increased. The change in alcohol content was influenced by differences in the initial sugar content that yeast and bacteria used for growth and fermentation activities (Dufresne and Farnworth, 2000; Sievers et al., 1995; Reiss, 1994). However, using starting sugar concentration of 2-8% for RD 43 kombucha fermentation provided alcohol content rapidly when compared to using starting sugar concentration of 10%. A similar result was obtained in a previous investigation of kombucha properties using a 10% sugar concentration for fermentation (Muhialdin et al., 2019). It was noticed that during 3 days of fermentation alcohol content increased rapidly because at the beginning of kombucha fermentation (day 0 – day 3) and total yeast count was higher than acetic acid bacteria (Katarzyna et al., 2017). The role of yeast in the fermentation process is to convert sugar into alcohol and carbon dioxide. According to acetic acid content, maximum acetic acid production was on day 6 and day 12 when using starting sugar concentrations of 10% and 2-8% respectively. This indicated the kombucha fermentation using starting sugar concentration of 10% was more quickly than that using other starting sugar concentrations. Similar results were reported by several researchers (Lannino et al., 1988; Sievers et al, 1995; Chen and Liu, 2000; Zhang et al., 2011). The increase of acetic acid reached the maximum level and then tended to be stable until the end of fermentation in accordance with the report of Katarzyna et al, (2017). They also showed that the main organic acid in kombucha was acetic acid which was found on the 3rd and the 10th day higher than the total yeast count. The role of acetic acid bacteria in the fermentation process was to metabolize alcohol produced by yeast into acetic acids and create a new biofilm of cellulose layers. RD 43 kombucha fermentation using 2-4% starting sugar concentration produced 0.3% acetic acid which was the same amount of acetic acid content in the commercial product (Noronha et al., 2022). Considering the biofilm thickness formation, the thickness increased as the starting sugar concentration increased. Malbasa et al. (1995) also reported that biomass yield during kombucha fermentation was closely related to sucrose levels. The high sugar level affected the high biomass utilization and synthesis.

After 15 days of fermentation, kombucha samples from different starting sugar concentrations were tested for consumer acceptance with 10 trained tasters using the 9-point hedonic scale method. Characteristics of kombucha samples were dark brown colored, little sparking, sour and unique aroma which were different among the samples. The result showed that aroma, taste, and overall liking of kombucha from starting sugar concentration of 4-10% were accepted in the level of moderately like (score = 7), followed by kombucha from 2% sugar which had the level score of slightly likes (score = 6) as shown in Table 1. It was noticed that kombucha starting with 0 °Brix was not accepted by the tasters because they evaluated the sample with the same score of 1 point.

**Table 1** Sensory evaluation of RD 43 kombucha from different initial sugar concentrations by 10 trained tasters

| **Attributes** | **Score** | | | | | |
|---|---|---|---|---|---|---|
| | **0%** | **2%** | **4%** | **6%** | **8%** | **10%** |
| Aroma | $1.00±0.00^{C}$ | $5.85±0.25^{B}$ | $6.93±0.20^{A}$ | $6.90±0.25^{A}$ | $6.99±0.28^{A}$ | $6.86±0.25^{A}$ |
| Taste | $1.00±0.00^{C}$ | $5.99±0.20^{B}$ | $6.95±0.26^{A}$ | $6.97±0.20^{A}$ | $7.16±0.29^{A}$ | $7.00±0.25^{A}$ |
| Overall liking | $1.00±0.00^{C}$ | $5.76±0.20^{B}$ | $6.93±0.27^{A}$ | $6.91±0.27^{A}$ | $7.15±0.30^{A}$ | $6.95±0.21^{A}$ |

*Note: The data are mean ± standard deviation with different superscripts in the same row showing statistically significant differences ($p \leq 0.05$).*

For commercial production, the use of 4% initial sugar concentration received the highest score in all attributes, thus this concentration was chosen for the production of low sugar kombucha. Aside from the sensory quality, another advantage of using 4% initial sugar was the low cost of the raw materials.

## 5. Conclusion

The chemical and sensory qualities of Kombucha fermentation from RD 43 wort depended on the initial sugar concentration. Using a low sugar level of 2-10%, the fermentation time was shortened to 9-15 days. This could reduce the potential for undesirable microbial contamination, a major problem in long-term kombucha fermentation. For the sensory test, it was found that kombucha fermented with an initial sugar concentration of 4 % had the same sensory acceptance score as kombucha fermented with a sugar concentration of 10% at the level of moderately like. Future studies should be carried out to determine and evaluate the metabolites in kombucha for selling points of health benefits.

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