

Ethanol Formation Profile of Tape Snacks Using GC-FID

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Abstract: This study profiles ethanol formation in traditional Indonesian tape snacks during seven consecutive days of spontaneous fermentation using Gas Chromatography–Flame Ionization Detection (GC-FID). Three rice tape samples (TB1–TB3) and four cassava tape samples (TU1–TU4) were obtained from different local producers and monitored daily (Day 1–7). Ethanol was quantified by external calibration using absolute ethanol standards. Across samples, ethanol concentrations increased during the early fermentation phase, reached a sample-dependent peak (generally on Day 3–6), and then decreased or stabilized toward Day 7. Cassava tape showed broader variability in peak ethanol levels (10.988–23.258 ppm) than most rice tape samples (5.578–29.249 ppm), reflecting differences in raw materials and microbial consortia among producers. These ethanol formation profiles provide an analytical basis for quality monitoring of tape products and may support future standardization and safety assessments.

Keywords: Cassava, Ethanol, GC-FID, Rice, Tape

INTRODUCTION

Fermentation is one of the oldest food processing techniques used by humans to improve the quality, safety, and shelf life of food and beverage products (Yan et al., 2024). The fermentation process involves the activity of microorganisms, especially yeast, bacteria, and molds, which utilize carbohydrates as an energy source and convert them into various metabolites such as organic acids, alcohol, and aromatic compounds. In alcoholic fermentation, yeasts such as *Saccharomyces cerevisiae* play a dominant role in converting glucose into ethanol and carbon dioxide through glycolysis and anaerobic fermentation (Mallari & Brogi, 2025). This process not only affects the chemical content but also contributes to the formation of the distinctive flavor and texture of the food products produced (Copetti, 2019).

In Indonesia, fermentation not only serves as a preservation method, but also forms part of the traditional culinary heritage. Various fermented food products such as tempeh, tape, brem, and tuak have become part of the consumption culture of the community (Pawiroharsono, 2007). Among these products, tape occupies an important position as a popular traditional snack with a high economic value in various regions. Tape can be made from various starchy ingredients such as glutinous rice, cassava, or sweet potatoes, which are fermented using yeast containing a mixture of microbes, resulting in a sweet taste, soft texture, and distinctive aroma that consumers love. In addition to producing a distinctive flavor, the tape fermentation process also produces ethanol as a major by-product (Jayanti et al., 2024). The ethanol content is important to study because it is related to food safety, product quality, and regulatory compliance. Several regulations in Indonesia, such as those issued by the Food and Drug Supervisory Agency (BPOM) and the Indonesian Ulema Council (MUI), set limits on the alcohol content of certain food products, especially those widely consumed by the public (Wibiksana & Sumiyati, 2024).

Tape, as a traditional fermented product, can be made from various raw materials. Rice tape generally has a stickier texture, an intense sweet taste, and a strong distinctive aroma. The higher starch and simple sugar content in glutinous rice compared to cassava or sweet potato can be an optimal substrate for the growth of fermentation microbes, especially ethanol-producing yeast (Siebenhandl et al., 2001). Meanwhile, cassava tape has a softer texture and higher water content, which can affect the fermentation rate and the composition of the metabolites produced (Halake & Chinthapalli, 2020). The tape-making process involves the use of tape yeast, which is a mixed inoculum containing yeasts such as *Saccharomyces cerevisiae*, molds such as *Amylomyces rouxii*, and lactic acid bacteria such as *Lactobacillus* sp. This combination of microorganisms works synergistically to break down starch into simple sugars (saccharification process) and convert it into ethanol and other metabolite products (fermentation process) (Gronchi et al., 2019). Differences in raw materials, yeast types, and production locations have the potential to produce variations in fermentation profiles, making it important to analyze products from different locations to obtain a comprehensive picture (Kirchmayr et al., 2017).

Fermentation dynamics is a study that describes changes in substrate, product, and microbial biomass concentrations over time. In tape fermentation, fermentation kinetics can be interpreted through daily changes in alcohol content as an indicator of yeast metabolic activity (Nielsen, 2018). This process generally goes through an adaptation phase (lag phase), an exponential phase (log phase), a stationary phase, and a decline phase. Each phase reflects the dynamics of the microbial population and the rate of sugar conversion to ethanol (Moimenta et al., 2024). Differences in substrate composition between rice tape and cassava tape can cause differences in fermentation kinetics patterns. Therefore, analysis of daily changes in alcohol content can provide an in-depth scientific description of the fermentation mechanism, while also helping to identify the optimum fermentation point (Berlian et al., 2016; Rofi'i et al., 2022).

To accurately analyze alcohol content, a sensitive and selective analysis method is required. Various methods have been used, ranging from conventional techniques such as distillation and spectrophotometry to modern instruments such as gas chromatography (GC). Conventional methods have limitations in precision and selectivity, especially when samples contain many volatile compounds. In contrast, GC is capable of specifically separating and measuring ethanol with high sensitivity, utilizing the principle of separating mixed components in a chromatography column and detection using a Flame Ionization Detector (FID) (Charapitsa et al., 2021). In the context of fermented food analysis, GC can measure ethanol levels along with other volatile compounds simultaneously, with short analysis times and reproducible results (Anghelescu et al., 2017).

Based on this, this study aims to quantify ethanol content in rice tape (TB) and cassava tape (TU) and to describe their ethanol formation profiles during seven consecutive days of spontaneous fermentation using GC-FID. The resulting profiles are expected to provide practical information for quality monitoring of traditional fermented foods.

RESEARCH METHODS

Materials and Tools

The materials used in this study were rice tape (TB) and cassava tape (TU) obtained from several local producers, standard ethanol solution ($\geq 99.9\%$, Merck), distilled water, Whatman No. 1 filter paper, and helium gas (99.99%, UHP grade) as the carrier gas.

Methods

The research procedure consisted of sample preparation, preparation of ethanol standard solutions, and analysis of alcohol content using gas chromatography (GC). Three rice tape samples (TB1, TB2, TB3) and four cassava tape samples (TU1, TU2, TU3, TU4) were collected on the first day of fermentation (H0). Each sample was left to undergo natural fermentation at room temperature (27–30°C) for seven consecutive days. Approximately 50 g of tape was taken every day at the same time (around 10:00 a.m. Western Indonesian Time), then homogenized using a laboratory blender and filtered with Whatman No. 1 filter paper to obtain a clear filtrate. This 7-day observation window was selected to capture the rising, peak, and post-peak phases of ethanol formation under typical room-temperature storage conditions.

Standard ethanol solutions were prepared by diluting absolute ethanol with distilled water to obtain concentrations of 0, 5, 10, 15, 20, and 25 ppm. Each standard solution was placed in a sterile GC vial and then injected into the GC under the same conditions as the samples. A calibration curve is created by plotting the ethanol concentration against the peak area to produce a linear regression equation used to calculate the ethanol concentration in the sample.

Alcohol content analysis was performed using Shimadzu GC-2010 Plus Gas Chromatography equipped with a Flame Ionization Detector (FID) and an Rtx-WAX capillary column (30 m × 0.25 mm × 0.25 µm). The GC operating conditions were as follows: injector temperature 200°C, detector temperature 250°C, column temperature isothermal at 40°C for 5 minutes, helium carrier gas flow rate 1 mL/minute, split ratio 50:1, and injection volume 1 µL. Each prepared extract was injected in duplicate (two repeated GC injections of the same extract), and the alcohol concentration was calculated based on the standard calibration curve equation. The reported ethanol concentration for each day/sample is the mean of the two injections.

RESULTS AND DISCUSSION

Tape is a traditional fermented product produced by breaking down starch into simple sugars, which are then converted into ethanol and other metabolites by microorganisms. At the beginning of fermentation (day 0), both rice tape (TB) and cassava tape (TU) show relatively low alcohol content. This is normal because in the early stages, the microorganisms contained in the tape yeast are still in the adaptation phase (lag phase), where metabolic activity is not yet optimal for producing ethanol.

The dominant microflora in tape yeast—mainly *Saccharomyces cerevisiae* as an ethanol producer, lactic acid bacteria such as *Lactobacillus* sp. which play a role in producing organic acids, and starch-hydrolyzing fungi—have an important contribution to the dynamics of fermentation (Albergaria & Arneborg, 2016). This combination of microbes works in stages: fungi break down starch into simple sugars, yeast converts sugars into ethanol and CO₂, while bacteria contribute to the formation of other compounds that affect aroma and flavor (Bhalla, 2017).

The initial fermentation conditions, as indicated by low alcohol content on the first day, are consistent with the fermentation patterns of other traditional starch-based foods. Previous studies have also shown that the alcohol content in tape products is generally still very low at the beginning of fermentation and begins to increase significantly after entering the second to fourth days of intensive fermentation (Widjajanti et al., 2017; Lestari & Utami, 2020). This confirms that the observations in this study are consistent with previous findings,

while also showing that the characteristics of the raw materials play an important role in determining the potential fermentation rate in the next stage.

The results of the alcohol content analysis shown in Table 1 and Figure 1 indicate a pattern of increasing ethanol content from the first day until it peaks in the middle of the fermentation period, then tends to stabilize or decrease in the following days. In rice tape (TB), the alcohol content increased sharply from the second day until it peaked on the third or fourth day of fermentation. Meanwhile, cassava tape (TU) showed a slower increase, with maximum ethanol content generally reached on the fourth or fifth day.

Table 1. Summary of ethanol formation profile parameters for all tape samples during 7 days of fermentation (based on daily GC-FID measurements)

Sample	Type	Peak day	Peak ethanol (ppm)	Mean ethanol (ppm)
TB1	Rice tape	4	5.578	4.429
TB2	Rice tape	3	29.249	19.983
TB3	Rice tape	5	6.981	5.467
TU1	Cassava tape	5	10.988	7.205
TU2	Cassava tape	4	14.646	10.174
TU3	Cassava tape	4	23.258	8.655
TU4	Cassava tape	6	15.524	7.805

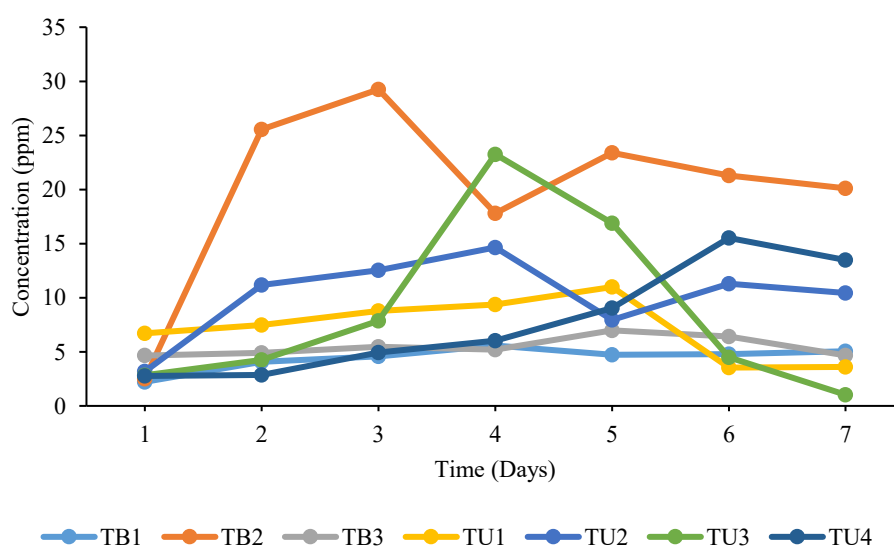


Figure 1. Ethanol formation profiles (daily ethanol concentration) in rice tape (TB1–TB3) and cassava tape (TU1–TU4) during 7 days of fermentation.

Figure 2 Representative GC-FID chromatograms of rice tape (TB2) and cassava tape (TU3) during fermentation, highlighting the ethanol peak (retention time ~0.64–0.70 min).

The chromatogram patterns between rice tape (TB) and cassava tape (TU) also show clear differences, as seen in Figure 2. In rice tape, the ethanol peak appears earlier with a relatively stable retention time (0.652–0.698 minutes) and the intensity increases sharply from day 2 to reach its peak on days 3 to 4. This condition is consistent with the quantitative data in Table 1, where rice tape shows a maximum ethanol content faster than cassava tape. Conversely, cassava tape displays a slightly wider retention time variation (0.647–0.682

minutes) with a sharp peak only visible on the 4th to 5th day, indicating a slower fermentation rate due to a more complex substrate composition. Representative chromatograms are displayed for TB2 and TU3 as examples; chromatograms from other samples/days showed the same ethanol peak position with different peak intensities.

The Gas Chromatography (GC) chromatogram results shown in the supplementary figure demonstrate the consistency of ethanol detection with retention times in the range of 0.643–0.698 minutes, very close to the ethanol standard of 0.684 minutes. This similarity indicates that the GC method used in the study has high selectivity and accuracy in separating and detecting ethanol from the complex tape matrix.

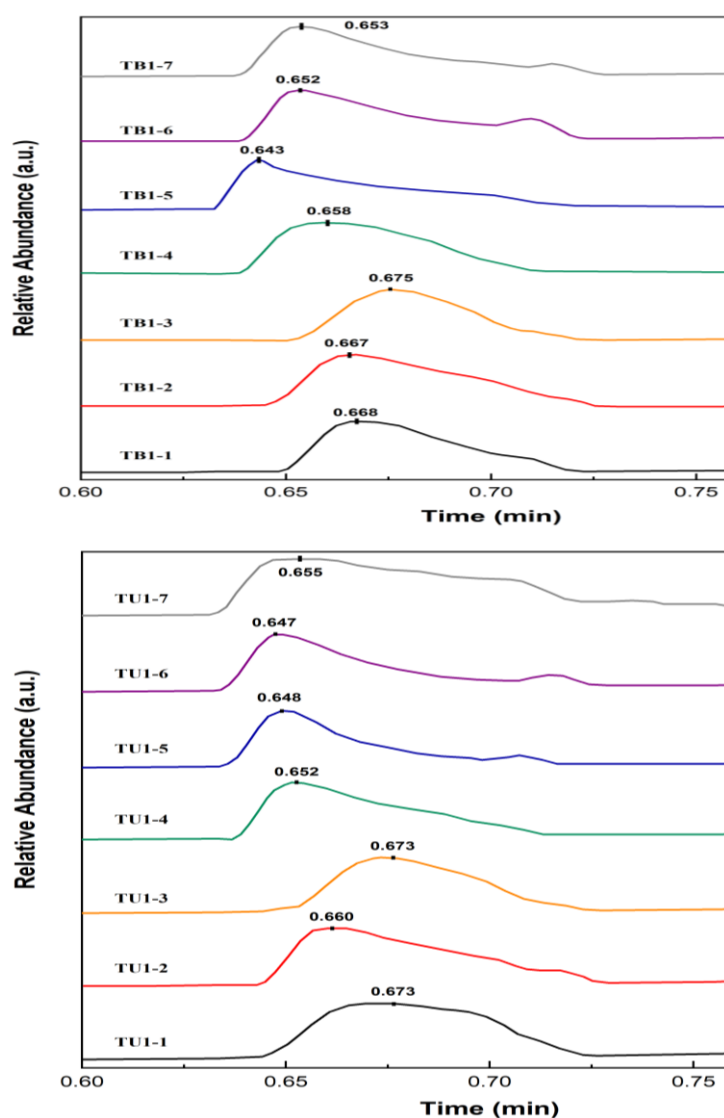


Figure 1. Chromatogram of GC Analysis Results on TB and TU Samples with Time Variations

The difference in the rate of increase in ethanol levels can be attributed to the composition of the available substrate. Rice tape, especially from glutinous rice, has a high starch and glucose content, providing a substrate that is more easily utilized by *Saccharomyces cerevisiae*. This allows ethanol production to occur more quickly in the early stages of fermentation. In contrast, cassava tape has a relatively lower simple sugar content and higher fiber content, so the process of converting starch into ethanol takes longer. These findings are in line with the research by Lestari and Utami (2020), which reported that rice-based tape produced higher ethanol levels than cassava tape during the same fermentation period.

In addition, variations in ethanol content between samples from different production locations (TB1–TB3, TU1–TU4) were also observed. This could be due to differences in the microbial composition of traditional tape yeast, the length of initial fermentation before samples were obtained, and environmental factors such as temperature and humidity of the storage location. According to Wardhani et al. (2019), the diversity of microflora in local tape yeast greatly affects the metabolite profile formed during fermentation, including ethanol production.

The decrease in ethanol levels at the end of the fermentation period (days 6 to 7) was likely caused by two main factors: (1) the ethanol produced evaporated due to open storage conditions, and (2) some of the ethanol was oxidized into acetic acid by oxidative microorganisms such as *Acetobacter* sp. This phenomenon was also reported by Widjajanti et al. (2017), who found that the alcohol content of tape decreased after the 5th day due to the conversion of ethanol into other organic compounds.

Thus, the results of this study indicate that rice tape has a faster and higher ethanol content increase profile compared to cassava tape, with significant variations between production locations. This confirms the importance of controlling raw materials and the fermentation process in determining the quality and alcohol content of traditional tape products.

The pattern of alcohol content changes in rice tape (TB) and cassava tape (TU) during seven days of fermentation shows dynamics that are in line with the principles of microbial fermentation dynamics. Daily analysis results show that the alcohol content increases gradually from the first day, then enters a rapid growth phase until it reaches its peak, before finally stabilizing or decreasing towards the end of the fermentation period.

Furthermore, the chromatogram profile (Figure 2) supports the interpretation of fermentation kinetics described above. On the first day, the peak intensity was still low, indicating a lag phase in which microorganisms adapted to the substrate. From the second to the fourth day, the peak increased sharply, describing an exponential phase with a high rate of sugar conversion to ethanol. After the peak is reached, especially on days 6 to 7, the peak intensity weakens, corresponding to the decline phase due to ethanol evaporation and oxidation to acetic acid by oxidative microbes.

The difference in kinetic patterns between rice tape and cassava tape is closely related to the composition of each substrate. Rice tape, with a higher glucose content, shows a faster fermentation rate and an earlier ethanol peak than cassava tape. Conversely, cassava tape requires more time because most of its carbohydrates are still in a complex form that needs to be hydrolyzed before being utilized by yeast. These results are in line with the research by Widjajanti et al. (2017), which reported that raw starch-based materials with higher simple glucose content will accelerate the fermentation rate and increase ethanol production.

CONCLUSIONS

The ethanol formation profiles of rice tape (TB1–TB3) and cassava tape (TU1–TU4) measured by GC-FID over seven days showed a typical rise to a sample-dependent peak followed by stabilization or decline toward the end of fermentation. Although rice tape could reach higher peak ethanol in one sample (TB2), cassava tape generally displayed broader variability across producers, indicating that raw material composition and microbial consortia likely influence ethanol formation. A limitation of this work is that replication was limited to duplicate GC injections and the microbial communities and fermentation conditions were not controlled or characterized in detail. Future studies should include biological replicates, controlled fermentation conditions, and complementary measurements (e.g., sugar/acid profiles and microbiological identification) to improve standardization and support safety-related evaluation of tape products.

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