CURRENT SCIENCE AND TECHNOLOGY
E-ISSN: 2785-8804
VOLUME 3, ISSUE 2, 2023, 7 – 12
DOI: https://doi.org/10.15282/cst.v3i2.10285

RESEARCH ARTICLE

Impact of Storage Temperature and Duration on Ethanol Content in Juice and Soft Drinks

S. S. Mohamed Shariff [1,2], N. H. A. Adnan[1] and N. Zaharudin[1]*

[1]Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuh Persiaran Tun Khalil Yaakob, 26300 Kuantan, Pahang, Malaysia

[2]Politeknik METrO Kuantan, No. A-5, Jln Tun Ismail 11, Sri Dagangan 2, 25000 Kuantan, Pahang, Malaysia

ABSTRACT - The ethanol content in beverages can be impacted by storage conditions, presenting potential implications for product quality and consumer safety. The study examined the impact of storage temperature and duration on ethanol levels in various beverages, including sugarcane juice, grape juice, and soft drinks. The findings suggest that storage conditions significantly influence ethanol production, particularly in sugarcane juice. Storing sugarcane juice at 28°C for 14 days resulted in a substantial increase in ethanol content, from 2.11% to 7.32% alcohol by volume (ABV). In contrast, grape juice exhibited moderate ethanol increases, and soft drinks showed minimal changes, with ethanol peaking at 0.95% ABV. These results highlight the accelerated fermentation process in sugarcane juice under higher temperatures and underline the importance of maintaining strict storage conditions to preserve beverage quality. Comparative analysis indicates that sugarcane juice is highly susceptible to ethanol generation when stored at room temperature, emphasizing the necessity of refrigeration to maintain its safety and flavor profile.

ARTICLE HISTORY

Received	:	01st May 2023
Revised	:	03rd July 2023
Accepted	:	07th Nov. 2023
Published	:	29th Dec. 2023

KEYWORDS

Ethanol
Gas chromatography
Beverages
Storage time
Temperature

1. INTRODUCTION

Alcohol refers to a class of organic compounds defined by the presence of a hydroxyl group (-OH) bonded to a carbon atom [1]. This broad class encompasses numerous compounds, of which ethanol is one of the most widely utilized. Ethanol can be naturally occurring or intentionally added to food and beverage products, where it serves to enhance flavor and used as preservation. Furthermore, ethanol is used in many applications, ranging from inclusion in alcoholic beverages to use as a solvent in laboratory and pharmaceutical products [2]. Moderate alcohol consumption has some social and health benefits, but excessive or chronic consumption poses serious health risks such as liver disease, addiction, and various types of cancer. It may also have negative effects on mental health and social interactions [3][4].

Among the available analytical techniques, gas chromatography with flame ionization detection (GC-FID) emerges as a precise, sensitive technique for quantifying ethanol in complex beverage matrices. GC-FID offers several advantageous features, including high resolution, specificity, and reproducibility, which make it a preferred choice for regulatory and quality control applications. The technique has been widely utilized for the analysis of ethanol content in alcoholic beverages, food products, and pharmaceutical preparations [5]-[7]. This study employs GC-FID to ensure accurate measurement of ethanol concentrations across varying conditions.

Beverages, whether they are refreshing fruit juices or other liquid delights, frequently change composition over time, especially when subjected to different storage conditions. Among the components that are susceptible to change is ethanol, which is a common and potentially volatile ingredient in some beverages. Maintaining consistent ethanol levels is crucial for food and beverage quality and safety. Ethanol levels in beverages can change due to storage conditions like temperature, light, and container type. These fluctuations can impact the taste and shelf life of beverages, and raise potential safety concerns, particularly for non-alcoholic products.

Previous research has shown that storage conditions can trigger chemical changes in beverages. A study by [8] revealed that temperature and light speed up chemical reactions, leading to changes in the amount of ethanol. To address this, the researchers stored the beverages at a moderate temperature (20°C) to minimize turbidity and stabilize volatile compounds. Another study by [2], demonstrated how byproducts from fermentation can cause ethanol levels to rise over time. Based on these findings, this study aims to examine how ethanol levels change in controlled experiments. This study investigates how different storage conditions specifically temperature and duration affect the ethanol content of selected beverages.

***CORRESPONDING AUTHOR | N. Zaharudin** | ✉ nazikussabah@umpsa.edu.my

2. METHODS AND MATERIAL

2.1 Materials and Chemicals

All chemicals that are the internal standard of ethanol, ethanol (≥99.89%), and acetonitrile that were used throughout the assays were purchased from Sigma-Aldrich, Darmstadt, Germany. Samples consisting of different beverages which are sugarcane, grape juice, and soft drinks were purchased from the local markets near Kuantan, Pahang, Malaysia.

2.2 Preparation of Standard Stock Solution for Ethanol and Acetonitrile

The ethanol standard solutions were prepared at 5 concentrations ranging from 0.1 to 1.6% v/v. The ethanol stock solution was prepared by adding 5 mL of pure ethanol and was diluted with distilled water using 100 mL of a volumetric flask. For acetonitrile stock solution, the previous step was repeated by using 5 mL of acetonitrile.

2.3 Preparation of Samples

The samples bought were kept in a glass jar and kept at room temperature (28°C) and in the refrigerator (8°C). The storage times and incubation periods were 1, 2, 3, 7, 14, 21, and 28 days. After the incubation time, a volume of 100 µL of sample was mixed with 1 mL of distilled water and 50 µL of acetonitrile and this preparation technique was a modification technique developed by [9]. Then, the solution was stirred continuously until it mixed well. After that, 1000 µL of micropipette was used to transfer the mixed solution into a GC vial for GC-FID analysis.

2.4 Determination of Ethanol Content

The quantitative analysis of ethanol was performed using a modification method by [9]. All samples were analyzed using Agilent Technologies 7890A GC system equipped with FID and an automatic sampler. The components were separated by using Agilent J&W HP-INNOWax column [30 m (L) x 0.25 mm (ID) x 0.25 µm film thickness] as a stationary phase. The injection volume of each sample was 1 µL in split mode with a split ratio of 20:1. The carrier gas used in the GC was Helium (He) at the flow rate of 1 mL/min whereas the flow rates of H_2 and air were set at 35 mL/min and 350 ml/min, respectively. The temperature of the FID detector and injection port was set at 223 ℃ and 250 ℃, respectively and the oven temperature was set initially at 45 C for 1 min, and then increased to the final temperature of 245°C in 1 min at the rate of 10°C/min.

3. RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of the ethanol internal standard at a retention time of 1.778 min. The second peak at a retention time of 1.801 min is acetonitrile as the internal standard. Acetonitrile is important in identifying the ethanol peak because it helps to ensure that the result is accurate and consistent despite any variations that may occur during pre-treatment or sample and standard injection.

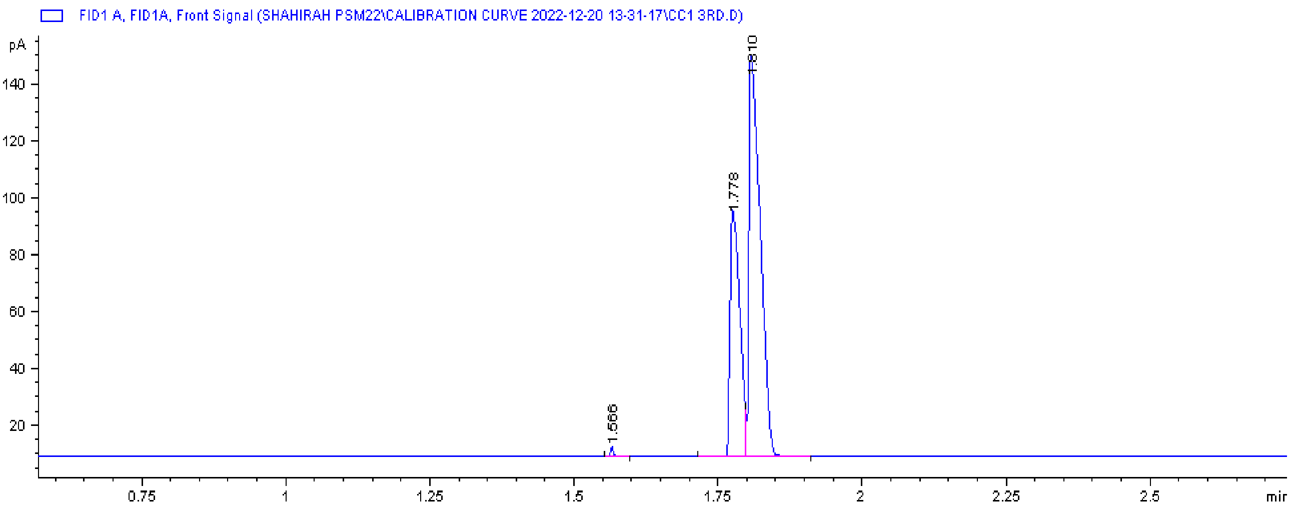


Figure 1. Chromatogram of ethanol internal standard (5 mg/ml) at 1.788 min and acetonitrile internal standard solution at 1.801 min

In this study, the ethanol content (% ABV - alcohol by volume) of sugarcane juice, grape juice, and soft drinks was monitored over a period of 28 days under two different temperature conditions refrigerated at 8°C and at room temperature, 28°C. As in Table 1, the ethanol content gradually increased from day 1 to 28.

Table 1. Ethanol content (% ABV) at 8°C and 28°C for 28 days in selected beverages

Sample	Temperature	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28
Sugarcane juice	Refrigerated (8°C)	2.08±0.58	2.87±0.45	4.81±0.32	5.40±0.36	5.72±0.21	5.72±0.20	5.73±0.23
	Room temperature (28°C)	2.11±0.25	4.62±0.58	6.15±0.23	6.90±0.33	7.32±0.13	7.31±0.10	7.29±0.12
Grape juice	Refrigerated (8°C)	0.01±0.00	0.20±0.08	0.66±0.09	0.77±0.05	0.90±0.06	0.77±0.03	0.71±0.01
	Room temperature (28°C)	0.16±0.02	0.64±0.05	1.13±0.11	1.76±0.08	1.76±0.43	1.68±0.10	1.92±0.06
Soft drink	Refrigerated (8°C)	0.16±0.04	0.40±0.06	0.47±0.08	0.58±0.06	0.75±0.05	0.61±0.07	0.59±0.07
	Room temperature (28°C)	0.14±0.01	0.44±0.03	0.58±0.08	0.82±0.02	0.88±0.11	0.95±0.12	0.78±0.06

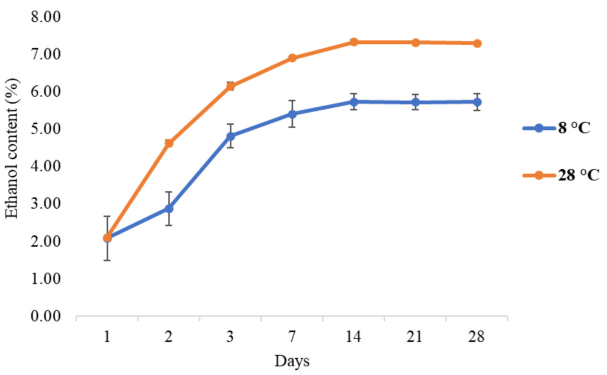
Across all beverage types, samples stored at room temperature showed a higher and faster increase in ethanol content than those kept refrigerated. When refrigerated, sugarcane juice had an ethanol content of 2.08±0.58% and gradually increased to 5.73±0.23%. At room temperature, there was a more pronounced rise from 2.11±0.25% to 7.29±0.12%, indicating that warmer conditions have a significant impact on fermentation. Grape juice, with lower initial ethanol levels, rose from 0.01±0.00% to 0.71±0.01% when refrigerated and from 0.16±0.02% to 1.92±0.06% at room temperature, indicating that even less fermentable beverages are susceptible to ethanol production over time. Soft drinks showed the smallest change in ethanol content, with refrigerated samples increasing from 0.16±0.04% to 0.59±0.07% and room temperature samples from 0.14±0.01% to 0.78±0.06%.

As in Figure 2, the graph presents the ethanol content in selected beverages measured over a period of 28 days at two different storage temperatures, refrigerated at 8°C and room temperature at 28°C. According to Figure 2 (a), at 8°C, the ethanol content in sugarcane juice begins at around 2.1% on day 1. It shows a consistent upward trend, reaching approximately 4.8% by day 3. The rate of increase slows down as the curve flattens slightly, with ethanol content reaching approximately 5.4% by day 7. The ethanol content gradually and consistently increases over the next two weeks, reaching around 5.7% by day 21. The content remains relatively stable, with a marginal increase that plateaus around 5.7% by day 28. At storage condition 28°C, around 2.1%, the sugarcane juice at room temperature shows a more rapid increase on day 1. By day two, the ethanol content had increased to nearly 4.6%, which was significantly higher than the refrigerated sample at the same point. The content continues to climb, reaching about 6.1% by day 3. The upward trend continues, but it becomes less steep with time. By day 14, the ethanol content is approximately 7.3%, and it continues to increase gradually, reaching about 7.3% by day 21. The increase slows, with the ethanol content stabilizing around 7.3% by day 28.

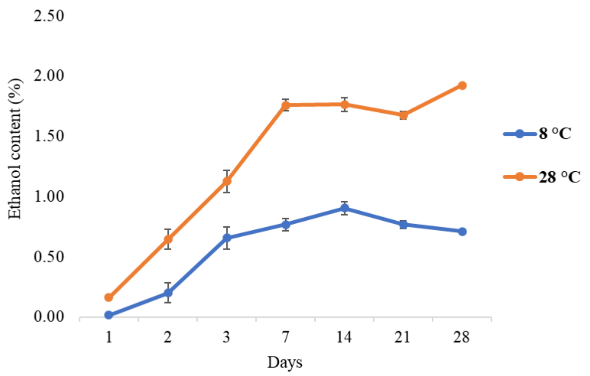
Grape juice stored at 8°C exhibits a gradual increase in ethanol content (Figure 2b). Starting from nearly 0% on day 1, there is a slight rise to just over 0.5% by day 3. This level is maintained with a very modest increase up to day 7. From day 7 to day 14, there is a slight fluctuation but essentially the ethanol content remains around 0.6%. Between day 14 and day 21, the ethanol content remains fairly stable, with a slight increase that continues through to day 28, ending at just below 0.8%. At room temperature, the grape juice shows a more pronounced increase in ethanol content (Figure 2b). From an initial level similar to the refrigerated sample, there is a notable rise to approximately 0.65% by day 2. A steep increase is observed by day 3, with ethanol content reaching around 1.3%. The content then continues to rise more gradually, reaching about 1.75% by day 7. Between day 7 and day 14, the ethanol content levels off slightly but then increases again, reaching around 1.9% by day 21. By day 28, the ethanol content appears to stabilize at approximately 1.9%.

According to Figure 2(c), the soft drink stored at 8°C has an initial ethanol content of approximately 0.16% on day one. The ethanol content gradually increases, reaching around 0.47% by day 7. After day 7, the ethanol content peaks around day 14 at just over 0.75%, before dropping slightly to around 0.61% on day 21. By day 28, the ethanol content has decreased to approximately 0.59%, whereas at room temperature (28°C), the ethanol content in the soft drink begins at a similar level to the refrigerated sample on day 1. It then steadily increases, peaking at 0.58% on day 7. The content continues to rise sharply, reaching a peak of approximately 0.88% by day 14. Following this peak, there is a noticeable drop by day 21 to around 0.95%, followed by another decrease to around 0.78% by day 28.

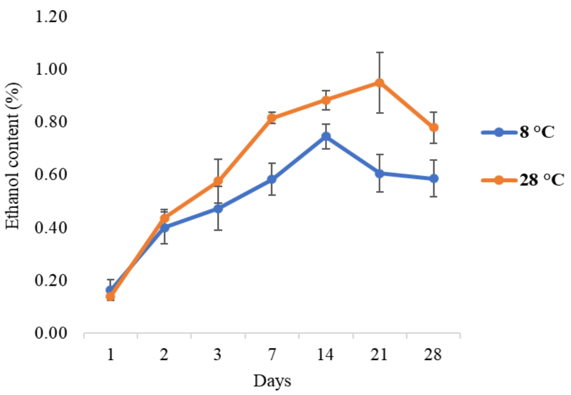
The experimental data presented in the table and figures show a clear relationship between storage temperature and ethanol content in sugarcane juice, grape juice, and soft drinks over a 28-day period. The ethanol content of sugarcane juice samples increased significantly at both temperatures, with fermentation occurring at room temperature (28°C) rather than refrigeration (8°C). This observation is consistent with the well-known principle that higher temperatures generally accelerate yeast metabolism and fermentation processes, leading to faster ethanol production [10]-[11]. The plateauing of ethanol content near the end of the observation period suggests that either yeast activity decreased due to an inhospitable environment caused by increased alcohol content or that the sugar available for fermentation was depleted and this was similar to a previous study done by other researchers [11]-[12]. The ethanol content in sugarcane-based beverages may also tend to increase due to the high fermentable sugar content such as sucrose, glucose, and fructose. These sugars are substrates for fermentation for yeast and bacteria to convert sugar to ethanol [14]-[16]. Grape juice samples also showed a trend toward higher ethanol content in warmer conditions. However, the overall ethanol concentration remained significantly lower than that of sugarcane juice, possibly due to the initial sugar content or the type of yeast present on the grape skins naturally or in the environment. The refrigerated grape juice kept a low ethanol level throughout the study, which is consistent with the common practise of storing wines in cool environments to slow down the fermentation and ageing processes [17].



(a)



(b)



(c)

Figure 2. Ethanol content (% ABV) at 8°C and 28°C for 28 days: (a) sugarcane juice (b) grape juice (c) soft drink

For soft drinks, the increase in ethanol content was the least pronounced among the three beverages tested. This could be due to the lower nutrient content available for yeast fermentation or the presence of preservatives that inhibit yeast growth. The peak and subsequent decline in ethanol content in room temperature samples could indicate a die-off of yeast populations or the exhaustion of fermentable substrates. The lower and more stable profile of ethanol content in refrigerated samples supports the industry standard of chilling soft drinks to maintain quality and flavor stability [18]. Soft drinks, which are more highly processed and contain preservatives, exhibited the smallest increase in ethanol levels over time, consistent with their intended stability and low risk of fermentation under normal storage conditions [19]. The increase in ethanol content in soft drinks was the least pronounced of the three beverages studied. This could be due to a lower nutrient content available for yeast fermentation or the presence of preservatives that prevent yeast growth. The peak and subsequent decline in ethanol content in room temperature samples may indicate yeast population death or the exhaustion of fermentable substrates. The lower and more stable profile of ethanol content in refrigerated samples supports the industry standard of chilling soft drinks to preserve quality and flavour stability [20].

4. CONCLUSION

In conclusion, storage temperature and duration significantly impact sugarcane juice with room temperature conditions favouring higher ethanol product compared with lower temperatures. Without refrigeration, sugarcane juice ferments more rapidly due to the activity. These findings provide information on optimizing parameters such as temperature to maintain product quality and extend shelf life to ensure consumer safety.

ACKNOWLEDGEMENTS

This research was supported by the Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Malaysia.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS CONTRIBUTION

N. Zaharudin (Conceptualization; Formal analysis; Visualisation; Supervision)

S. S. Mohamed Shariff (Introduction, Methodology, Data curation, Discussion)

N. H. A. Adnan (Introduction, Methodology, Discussion)

REFERENCES

[1] S. K. Tulashie, A. P. Appiah, G. D. Torku, A. Y. Darko, and A. Wiredu, "Determination of methanol and ethanol concentrations in local and foreign alcoholic drinks and food products (Banku, Ga kenkey, Fante kenkey and Hausa koko) in Ghana," *International Journal of Food Contamination*, vol. 4, no. 1, pp. 1-5, 2017.

[2] R. Gao, L. Xiong, M. Wang, F. Peng, H. Zhang, and X. Chen, "Production of acetone-butanol-ethanol and lipids from sugarcane molasses via coupled fermentation by Clostridium acetobutylicum and oleaginous yeasts," *Industrial Crops and Products*, vol. 185, p. 115131, 2022.

[3] J. Yang, L. Zhang, T. Wang, J. Zhang, M. Li, X. Jin, et al., "Synergistic effects of combined treatment of 1,2-dichloroethane and high-dose ethanol on liver damage in mice and the related mechanisms," *Food and Chemical Toxicology*, vol. 176, p. 113812, 2023.

[4] M. L. Martel, L. R. Klein, A. J. Lichtenheld, A. M. Kerandi, B. E. Driver, and J. B. Cole, "Etiologies of altered mental status in patients with presumed ethanol intoxication," *American Journal of Emergency Medicine*, vol. 36, no. 6, pp. 1057–1059, 2018.

[5] F. Wan, S. Liu, L. Wang, and S. Si, "A novel salting-out extraction system for determination of ethylene glycol and diethylene glycol in liquid samples followed by GC-FID," *Microchemical Journal*, vol. 179, p. 107491, 2022.

[6] C. L. dos Santos Costa, D. P. Ramos, and J. B. da Silva, "Multivariate optimization and validation of a procedure to direct determine acetonitrile and ethanol in radiopharmaceuticals by GC-FID," *Microchemical Journal*, vol. 147, pp. 654–659, 2019.

[7] C. K. Attchelouwa, F. K. N'guessan, S. Marcotte, T. L. S. Amoikon, M. Charmel, and M. K. Djè, "Characterisation of volatile compounds associated to sensory changes during the storage of traditional sorghum beer by HS-GC/FID and SPME-GC/MS," *Journal of Agriculture and Food Research*, vol. 2, p. 100088, 2020.

[8] M. Różański, K. Pielech-Przybylska, and M. Balcerek, "Influence of alcohol content and storage conditions on the physicochemical stability of spirit drinks," *Foods*, vol. 9, no. 9, p. 1264, 2020.

[9] A. R. Mansur, J. Oh, H. S. Lee, and S. Y. Oh, "Determination of ethanol in foods and beverages by magnetic stirring-assisted aqueous extraction coupled with GC-FID: A validated method for halal verification," *Food Chemistry*, vol. 366, p. 130526, 2022.

[10] M. Yilmaztekin, T. Cabaroglu, and H. Erten, "Effects of fermentation temperature and aeration on production of natural isoamyl acetate by williopsis saturnus var. saturnus," *BioMed Research International*, vol. 2013, p. 870802, 2013.

[11] H. T. Kim, E. J. Yun, D. Wang, J. H. Chung, I. G. Choi, and K. H. Kim, "High temperature and low acid pretreatment and agarase treatment of agarose for the production of sugar and ethanol from red seaweed biomass," *Bioresource Technology*, vol. 136, pp. 582–587, 2013.

[12] A. F. Danelon, H. F. S. Spolador, and J. S. Bergtold, "The role of productivity and efficiency gains in the sugar-ethanol industry to reduce land expansion for sugarcane fields in Brazil," *Energy Policy*, vol. 172, p. 113327, 2023.

[13] M. L. Cazetta, M. A. P. C. Celligoi, J. B. Buzato, and I. S. Scarmino, "Fermentation of molasses by Zymomonas mobilis: Effects of temperature and sugar concentration on ethanol production," *Bioresource Technology*, vol. 98, no. 15, pp. 2824–2828, 2007.

[14] F. Mehdi, S. Galani, K. P. Wickramasinghe, P. Zhao, X. Lu, X. Lin et al., "Current perspectives on the regulatory mechanisms of sucrose accumulation in sugarcane," *Heliyon*, vol. 10, no. 5, p. e27277, 2024.

[15] M. Aghbashlo, M. Tabatabaei, and K. Karimi, "Exergy-based sustainability assessment of ethanol production via Mucor indicus from fructose, glucose, sucrose and molasses," *Energy*, vol. 98, pp. 240–252, 2016.

[16] J. yil Park, E. Kanda, A. Fukushima, K. Motobayashi, K. Nagata, M. Kondo, et al., "Contents of various sources of glucose and fructose in rice straw, a potential feedstock for ethanol production in Japan," *Biomass Bioenergy*, vol. 35, no. 8, pp. 3733–3735, 2011.

[17] J. Echave, M. Barral, M. Fraga-Corral, M. A. Prieto, and J. Simal-Gandara, "Bottle aging and storage of wines: A review," *Molecules*, vol. 26, no. 3, p. 713, 2021.

[18] S. K. Amit, M. M. Uddin, R. Rahman, S. M. R. Islam, and M. S. Khan, "A review on mechanisms and commercial aspects of food preservation and processing," *Agriculture & Food Security*, vol. 6, pp. 1-22, 2017.

[19] S. Madhania, Y. Muharam, S. Winardi, and W. W. Purwanto, "Mechanism of molasses–water mixing behavior in bioethanol fermenter. Experiments and CFD modeling," *Energy Reports*, vol. 5, pp. 454–461, 2019.

[20] R. Sharma, P. Garg, P. Kumar, S. K. Bhatia, and S. Kulshrestha, "Microbial fermentation and its role in quality improvement of fermented foods," *Fermentation*, vol. 6, no. 4, p. 106, 2020.