

Original Research Article

Impact of Thermal Conditions on pH During Kombucha Fermentation

ABSTRACT

Kombucha is a beverage that undergoes fermentation. The intake of it has markedly risen in recent decades owing to its purported advantageous benefits. Consequently, it has evolved into a heavily marketed beverage that is mass-produced. Acidity, which is determined by pH, plays a crucial role in the process of fermentation. The objective of this research was to ascertain the variations in pH levels during fermentation at various temperatures and to find the optimal temperature for ensuring safe consumption. In order to achieve this objective, kombucha samples were fermented using green tea at temperatures of 20, 24, 28, and 32°C for a duration of 16 days. pH measurements were taken at a frequency of every 4 days. It was concluded that the pH range between 24 and 28°C was the most favorable for optimal fermentation and consumption.

Keywords: Kombucha, fermentation, pH, green tea, beverage

1. INTRODUCTION

Kombucha is a traditional fermented tea that has gained popularity in recent years owing to the possible health advantages that include its use. It has a high concentration of organic acids, minerals, vitamins, proteins, polyphenols, and other components that are helpful to the body. The antioxidant, antibacterial, and antifungal actions of kombucha are brought about by these substances, which contribute to the different medicinal qualities of kombucha [1, 2]. Kombucha has been proven to have the capacity to inhibit the growth of cancer cells and may also increase resistance to the disease. Research conducted by Mousavi et al. in 2020 discovered that the beverage had hepatoprotective properties [3].

According to Nizioł-Lukaszewska et al. [4], the fermentation process of kombucha requires a symbiotic culture of bacteria and yeast, which is often referred to as SCOBY. According to de Miranda et al. [5], the fermentation process results in the creation of bioactive compounds, such as antioxidants, which contribute to the health benefits of kombucha by contributing to the production of these compounds. The phenolic chemicals contained in kombucha are primarily responsible for the significant antioxidant activity that has been discovered in this beverage. According to Xiong et al. [6], the purported health advantages of the beverage may be attributed to the presence of an abundance of phenolic chemicals. A number of organic acids, including as acetic, malonic, glucuronic, gluconic, pyruvic, tartaric, citric, L-lactic, oxalic, succinic, usnic, malic and D-saccharic acid-1,4-lactone, have been shown to be present in kombucha, according to study that appeared in the past [7, 8, 9]. The pH level is important due to the organic acids contained in Kombucha.

Also, research has been done to determine whether or not kombucha has the capacity to act as a probiotic. Based on the findings of Kaashyap et al. [10], it has been discovered to possess a wide variety of microorganisms, which includes probiotic bacteria and yeasts. According to Kaashyap et al. [10], these bacteria have been shown to have positive effects on the health of the human gut, which suggests that kombucha has the potential to function as an effective probiotic. Due to the presence of probiotics in kombucha, it has the ability to improve both the health of the gut and the general well-being of the individual.

However, it is essential to keep in mind that while kombucha may have a number of possible advantages for one's health, there are also hazards linked with the intake of this beverage. According to Kim and Adhikari's research from 2020, there have been reports of a number of health dangers and adverse impacts, including the existence of biological and chemical threats. As a result, it is essential to guarantee the quality and safety of kombucha products by implementing appropriate production procedures and food safety precautions [11, 9, 12].

The purpose of this research was to ascertain whether or not the pH levels of kombucha samples that had been fermented with green tea at varying temperatures at the conclusion of the fermentation period are appropriate for ingestion.

2. MATERIAL AND METHODS

2.1 Kombucha Preparation

Green tea leaves and starter culture were collected from local shop in the Türkiye. The green tea broth was prepared by adding 7 g of green tea leaves to boiling water. After 30 min, the tea leaves were sieved and 70g of sucrose was added to prepare sugared green tea. Then, 1000 mL of sugared green tea was poured into a 2000 mL bottle, a 10% (v/v) starter culture was added. The bottle was covered with a cloth sheet and secured with rubber bands, then it was incubated for 16 days under aerobic conditions at temperatures ranging from 20°C to 32°C. pH values were measured and recorded every 4 days. The measurements were made in 3 parallels and averaged.

2.2 Statistical Analysis

The study reported the findings of multiple analyses using the mean \pm standard deviation format. The ANOVA was used to assess the means of the experimental results, and significance tests were conducted using Duncan's multiple range tests for statistical significance ($p < 0.05$).

3. RESULTS AND DISCUSSION

The purpose of this investigation was to ascertain the impact that variations in temperature have on the pH levels that occur during the fermentation process of kombucha. The fermentation procedure was carried out for a period of 16th days. Each of the pH values that were measured as a consequence of the fermentation process are shown in Table 1.

Table 1. pH values measured during the fermentation period

Kombucha Fermentation	0 th day	4 th day	8 th day	12 th day	16 th day
Kombucha at 20°C	6.74 \pm 0.12 ^a	6.35 \pm 0.08 ^a	6.01 \pm 0.04 ^a	5.57 \pm 0.04 ^a	5.31 \pm 0.08 ^a
Kombucha at 22°C	6.74 \pm 0.12 ^a	6.21 \pm 0.07 ^a	5.89 \pm 0.05 ^a	5.06 \pm 0.04 ^a	4.61 \pm 0.07 ^b
Kombucha at 24°C	6.74 \pm 0.12 ^a	5.99 \pm 0.08 ^b	5.10 \pm 0.05 ^b	4.23 \pm 0.05 ^b	3.74 \pm 0.06 ^c
Kombucha at 26°C	6.74 \pm 0.12 ^a	5.87 \pm 0.06 ^b	4.92 \pm 0.07 ^c	3.95 \pm 0.05 ^b	3.45 \pm 0.07 ^c

Commented [H1]: I don't think "starter culture" is the right term. What is purchased is a living cellulosic substrate known as a tea mushroom or SCOBY, which contains a large number of unidentified yeasts and bacteria.

Commented [H2]: In statistics, letters are assigned starting from the smallest numerical value.

Kombucha at 28°C	6.74±0.12 ^a	5.80±0.08 ^b	4.90±0.06 ^c	3.85±0.03 ^b	3.30±0.07 ^c
Kombucha at 30°C	6.74±0.12 ^a	5.74±0.07 ^c	4.81±0.05 ^c	3.72±0.03 ^b	3.01±0.06 ^c
Kombucha at 32°C	6.74±0.12 ^a	5.70±0.05 ^c	4.69±0.04 ^c	3.48±0.04 ^c	2.87±0.05 ^d

*The values shown are the average (mean) values with the standard deviation (S.D.) calculated from three separate measurements conducted in parallel. Distinct letters indicate statistically significant differences between the examined samples ($p < 0.05$).

The changes in pH that occur during fermentation are outlined in Table 1, which is based on the temperature. During the 14th day of fermentation at a temperature of 20°C, the pH value was measured to be 5.31±0.08. The pH value that was achieved was the maximum possible value. Having a pH value of this is acceptable for ingestion. However, it is not enough to guarantee that the fermentation of kombucha will have been completed. In addition, at a temperature of 32°C, the pH value was determined to be 2.87 with a standard deviation of 0.05. Among all of the samples, this particular pH value is the lowest. Consumption of kombucha should be avoided because of this value. According to Vitas et al. [13], kombucha samples that are ingested at this pH level are known to potentially cause injury to the human stomach.

The pH values obtained at 24-28 degrees Celsius on the 16th day were determined as 3.74±0.06, 3.45±0.07 and 3.30±0.07, respectively. These results are considered as the optimum pH range for kombucha consumption. Hammel et al. [14] tried to determine the optimum temperature for kombucha in their study. In this study, they kept black tea at 20 degrees Celsius for 5 days for kombucha fermentation. As a result, they found the pH values as 3.08 at the end of 5 days. Hammel et al. [14] suggested the optimum temperature as 20 degrees Celsius in their study. In kombucha samples prepared with black tea, a greater pH decrease can be seen during the fermentation period compared to green tea. This result differs from the kombucha samples we prepared using green tea. The reason for this is thought to be that the secondary metabolites found in black tea are higher than green tea.

Commented [H3]: °C

Deghrigue et al. [15] conducted fermentation for kombucha at a temperature of 25°C using the fermentation process. During the fermentation of black tea into kombucha, Fu et al. [16] used a temperature of 30°C. On day 8th, they decided to stop the fermenting process. They obtained the ideal pH level after a period of 8 days. For kombucha that is traditionally produced, the optimal amount of time for fermentation is fourteen days. On the eighth day, they decided to stop the fermentation process since they had attained a pH level that was suitable for internal consumption. On the other hand, they did not make it to the 14th day, which is the amount of time that is expected for the fermentation of kombucha. As a result, the pH value dropped significantly at a temperature of 30°C. The findings of this investigation are consistent with the findings that we obtained in this particular area. When conducting their research, Gaggia et al. [17] used a temperature of 28°C for the fermentation process. On the 7th and 14th day, they took pH readings. The result that they received demonstrates that the fermentation of kombucha proceeded without any problems. The pH readings that were obtained at the temperatures that were employed in the research are consistent with the findings that we obtained.

The fermentation of kombucha takes place at a variety of temperatures as a consequence. The pH levels that are appropriate for human ingestion, on the other hand, change depending on the findings that were obtained. It has been stated that the optimal pH range for consuming kombucha is between 3.74 and 3.30. This has to be taken into consideration. This pH range is reachable at temperatures of 24, 26, and 28°C, as shown by our findings.

4. CONCLUSION

In conclusion, kombucha is a beverage that is fermented and is eaten by a large number of individuals. Within the context of the ingestion of this fermented beverage, pH is a significant factor for the human stomach. It was discovered via the data that we received that the ideal temperature for kombucha, which is ready to be consumed, is between 24 and 28°C. This was discovered as a consequence of the fermentation process that lasted for fourteen days. The findings that we acquired were helpful in gaining preliminary information that revealed the significance of kombucha ingestion with regard to the pH of the human stomach. The findings of this study have made a contribution to the existing body of knowledge on the potential adverse effects that consuming kombucha samples within this pH range may have on the health of the human stomach. In order to get more specific information, it is suggested that research be carried out with the participation of human beings.

REFERENCES

1. Gülhan MF. A new substrate and nitrogen source for traditional kombucha beverage: stevia rebaudiana leaves. *Applied Biochemistry and Biotechnology*. 2023;195(7): 4096-4115.
2. Anantachoke N, Duangrat R, Sutthiphatkul T, Ochaikul D, Mangmool S. Kombucha Beverages Produced from Fruits, Vegetables, and Plants: A Review on Their Pharmacological Activities and Health Benefits. *Foods*. 2023;12(9): 1818.
3. Mousavi SM, Hashemi SA, Zarei M, Gholami A, Lai CW, Chiang WH, Omidifar N, Bahrani S, Mazraedoost S. Recent progress in chemical composition, production, and pharmaceutical effects of kombucha beverage: A complementary and alternative medicine. *Evidence-Based Complementary and Alternative Medicine*. 2020;2020: 1-14.
4. Nizioł-Lukaszewska Z, Ziemlewska A, Bujak T, Zagórska Dziok M, Zarębska M, Hordyjewicz Baran Z, Wasilewski T. Effect of fermentation time on antioxidant and anti-ageing properties of green coffee Kombucha ferments. *Molecules*. 2020;25(22): 5394-5421.
5. de Miranda JF, Ruiz LF, Silva CB, Uekane TM, Silva KA, Gonzalez AGM, Fernandes FF, Lima AR. Kombucha: A review of substrates, regulations, composition, and biological properties. *Journal of Food Science*. 2022;87(2): 503-527.
6. Xiong R-G, Wu S-X, Cheng J, Saimaiti A, Liu Q, Shang A, Zhou D-D, Huang S-Y, Gan R-Y, Li H-B. Antioxidant activities, phenolic compounds, and sensory acceptability of Kombucha-Fermented beverages from bamboo leaf and mulberry leaf. *Antioxidants*. 2023;12(8): 1573-1581.
7. Tu C, Yu T, Feng S, Xu N, Massawe A, Shui S, Zhang B. Dynamics of microbial communities, flavor, and physicochemical properties of kombucha-fermented *Sargassum fusiforme* beverage during fermentation. *LWT*. 2024;192: 115729.
8. Ojo AO, de Smidt O. Microbial composition, bioactive compounds, potential benefits and risks associated with kombucha: A concise review. *Fermentation*. 2023;9(5): 472.
9. Ziemlewska A, Zagórska-Dziok M, Nizioł-Lukaszewska Z, Kielar P, Mołoń M, Szczepanek D, Sowa I, Wójciak M. *In vitro* evaluation of antioxidant and protective potential of kombucha-fermented black berry extracts against H₂O₂-induced oxidative stress in human skin cells and yeast model. *International Journal of Molecular Sciences*. 2023;24(5): 4388.
10. Kaashyap M, Cohen M, Mantri N. Microbial diversity and characteristics of kombucha as revealed by metagenomic and physicochemical analysis. *Nutrients*. 2021;13(12): 4446-4460.

11. Kim J, Adhikari K. Current trends in kombucha: Marketing perspectives and the need for improved sensory research. *Beverages*. 2020;6(1): 15-34.
12. Zofia N-L, Aleksandra Z, Tomasz B, Martyna Z-D, Magdalena Z, Zofia H-B, Tomasz W. Effect of fermentation time on antioxidant and anti-ageing properties of green coffee Kombucha ferments. *Molecules*. 2020;25(22): 5394.
13. Vitas JS, Cvetanović AD, Mašković PZ, Švarc-Gajić JV, Malbaša RV. Chemical composition and biological activity of novel types of kombucha beverages with yarrow. *Journal of Functional Foods*. 2018;44: 95-102.
14. Hammel R, Karakilic V, Shaw F. The affect of temperature and pH on the food safety of kombucha tea. *BCIT Environmental Public Health Journal*. 2016;1: 1-9.
15. Deghrigue M, Chriaa J, Battikh H, Kawther A, Bakhrouf A. Antiproliferative and antimicrobial activities of kombucha tea. *African Journal of Microbiology Research*. 2013;7(27): 3466-3470.
16. Fu C, Yan F, Cao Z, Xie F, Lin J. Antioxidant activities of kombucha prepared from three different substrates and changes in content of probiotics during storage. *Food Science Technology*. 2014;34(1): 123-126.
17. Gaggia F, Baffoni L, Galiano M, Nielsen D, Jakobsen R, Castro-Mejía J, Bosi S, Truzzi F, Musumeci F, Dinelli G. Kombucha beverage from green, black and rooibos teas: a comparative study looking at microbiology, chemistry and antioxidant activity. *Nutrients*. 2019;11(1): 1-22.