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An Investigation of the Impact of Fermentation Conditions and Total Acid Contents on the Kombucha Tea

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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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. Kombucha is characterised by rich chemical content and healthy properties. It includes organic acids, minerals and vitamins originating mainly from tea, amino acids, and biologically active compounds—polyphenols in particular. 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.

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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]. Kombucha is very rich in various bioactive compounds, including acetic and gluconic acid, complex B vitamins, minerals, amino acids, and polyphenols, that have been recognized as having health benefits, such as anti-inflammatory, antioxidant, antidiabetic, and antimicrobial activity [4]. The literature reports the impact of the biological properties of Kombucha on health and several disorders, such as inflammatory diseases, arthritis, allergies, cardiovascular diseases, and cancer [5].

According to Nizioł-Łukaszewska et al. [6], 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. [7], 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. [8], 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 [9,10,11]. 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. [12], it has been discovered to possess a wide variety of microorganisms, which includes probiotic bacteria and yeasts. According to Kaashyap et al. [12], 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 [13,11,14].

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. Moreover, it is to determine the amount of total acidity level.

2. MATERIALS AND METHODS

2.1 Kombucha Culture Preparation

The kombucha starter cultures, also known as SCOBY and green tea (*Camelia sinensis*) leaves were purchased 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 [15].

2.2 Fermentation of Kombucha

Kombucha cultures were kept under aseptic conditions in a climate cabinet that maintained constant temperature and humidity. Fermentation

was carried out by incubating the kombucha culture at temperatures ranging from 20°C to 32°C for 4, 8, 12 and 16 days. Replicates were prepared so that each replicate was completely collected after its stipulated period of fermentation. The kombucha obtained was filtered and analysed [16].

2.3 pH Determination

pH measurement was performed using a pH meter in 3 repetitions every 4 days (WTW series Multi 9620 IDS, Weilheim, Germany).

2.4 Total Acidity

The measurement of total acidity was conducted using titration, using a standard solution of 0.1 mol/L sodium hydroxide and phenolphthalein as an indicator. The results were quantified in terms of grams of acetic acid per liter of sample [17].

2.5 Statistical Analysis

The study reported the findings of multiple analyses using the mean ± 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.

The changes in pH that occur during fermentation are outlined in Table 1, which is based on the temperature. During the 16th 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. [18], 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°C 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. [19] studied to determine the optimum temperature for kombucha in their study. In this study, they kept black tea at 20°C 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. [19] suggested the optimum temperature as 20°C 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.

Table 1. pH values measured during the fermentation period

Kombucha Fermentation	0th day	4th day	8th day	12th day	16th day
Kombucha at 20℃	6.74±0.12^{a}	6.35±0.08^{c}	6.01±0.04^{c}	5.57±0.04^{c}	5.31±0.08^{d}
Kombucha at 22℃	6.74±0.12^{a}	6.21±0.07^{c}	5.89±0.05^{c}	5.06±0.04^{c}	4.61±0.07^{c}
Kombucha at 24℃	6.74±0.12^{a}	5.99±0.08^{b}	5.10±0.05^{b}	4.23±0.05^{b}	3.74±0.06^{b}
Kombucha at 26℃	6.74±0.12^{a}	5.87±0.06^{b}	4.92±0.07^{a}	3.95±0.05^{b}	3.45±0.07^{b}
Kombucha at 28℃	6.74±0.12^{a}	5.80±0.08^{b}	4.90±0.06^{a}	3.85±0.03^{b}	3.30±0.07^{b}
Kombucha at 30℃	6.74±0.12^{a}	5.74±0.07^{a}	4.81±0.05^{a}	3.72±0.03^{b}	3.01±0.06^{b}
Kombucha at 32℃	6.74±0.12^{a}	5.70±0.05^{a}	4.69±0.04^{a}	3.48±0.04^{a}	2.87±0.05^{a}

**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 ($p<0.05$)*

Table 2. Total acidity level during the fermentation period

	Total acidity (g acetic acid/L)				
Kombucha Fermentation	**0th day**	**4th day**	**8th day**	**12th day**	**16th day**
Kombucha at 20°C	0.00±0.00[a]	1.53±0.07[a]	3.01±0.05[a]	5.07±0.04[a]	7.31±0.08[a]
Kombucha at 22°C	0.00±0.00[a]	0.91±0.06[a]	2.79±0.05[a]	5.06±0.04[a]	7.71±0.07[a]
Kombucha at 24°C	0.00±0.00[a]	2.31±0.07[b]	5.21±0.05[b]	8.43±0.06[b]	10.64±0.04[c]
Kombucha at 26°C	0.00±0.00[a]	2.45.±0.05[b]	5.32±0.06[b]	8.91±0.04[b]	11.45±0.05[c]
Kombucha at 28°C	0.00±0.00[a]	2.39±0.07[b]	5.25±0.05[b]	8.64±0.04[b]	11.32±0.06[c]
Kombucha at 30°C	0.00±0.00[a]	1.87±0.06[a]	3.81±0.05[a]	5.42±0.03[a]	9.01±0.06[b]
Kombucha at 32°C	0.00±0.00[a]	1.91±0.04[a]	3.69±0.04[a]	5.48±0.04[a]	9.78±0.05[b]

**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 ($p<0.05$)*

Deghrigue et al. [20] 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. [21] 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 14 days. On the 8th 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 16th 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, Gaggìa et al. [22] used a temperature of 28°C for the fermentation process. On the 7th and 16th 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. Neffe-Skocińska et al. [23] used temperature 20°C, 25°C and 30°C for the pH change. It was reported that pH change was 2.67, 2.77 and 2.63, respectively for the 10 days fermentation. These findings shows accordance with our results. They stated that optimal conditions for the fermentation of Kombucha beverages were a temperature of 25°C and a period of 10 days which allowed to obtain a microbiologically stable product. Moreover, they stated that value of pH and total acidity were standard parameters that indicate the success of the production process [24]. The amount of the herb also influenced the fermentation in the way that higher amount caused intensification of the process [18].

This can be explained either by the cell concentration of acetic acid bacteria or by the metabolic path followed in each culture media, considering the hypothesis that some bacteria can produce ethanol and others can consume it and produce acetic acid. This suggests that important interactions occur between yeast and bacteria that are present both in the liquid broth and in the cellulose pellicle [25,26].

Total acidity change findings are given in Table 2. During the 16th day of fermentation at temperature of 20°C, the total acidity was measured to be 7.31±0.08 at the lowest concentration. Moreover, the highest total acidity was determined as 11.45±0.05 at temperature of 26°C on the 16th day of fermentation process. Additionally, total acidity measurements at 24 and 28°C were found to be 10.64±0.04 and 11.32±0.06, respectively. These results were similar to at temperature of 26°C. it is not statistically significant with It was not found to be statistically significant according to the results at 26°C.

Total acidity is also an usual parameter of kombucha fermentation that indicates the metabolic activity of used starter [5]. Kombucha culture caused the gradual increase in total acidity during fermentation, for all beverages. The biggest difference in total acids content was between initial substrates and the start of fermentation for samples with black tea and green tea. The significant increase of total acidity was noticed between start of fermentation and 4th day for all samples. The same trend was detected in all samples until the end of the fermentation period. In their study, Jakubczyk et al. [16], prepared green and black kombucha

samples and subjected them to fermentation at 30°C for 14 days and measured their total acidity. After 14 days of fermentation, they found total acidity to be 9.14 for green tea and 9.08 for black tea. In similar studies, the acidity of kombucha tea was reported to be around 8 g/L on the 10th fermentation day [27,28]. The 16th day of fermentation was shown to have greater acidity levels than these studies in this study. A study on the effects of the Kombucha tea fermentation period found that the kombucha tea's acidity level was approximately 12 g/L on the 15th day of fermentation and between 15 g/L and 16 g/L on the 21st day of fermentation [29]. The results they obtained are parallel to our results. As a result, fermentation temperature is thought to be effective on organic acid production. In addition, it is thought that the phenolic compounds contained in green or black tea used in the preparation of kombucha also have an effect on organic acid production.

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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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