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The Effect of Storage Conditions on Ethanol Content of Kombucha Tea

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ABSTRACT

Ethanol is one of the alcohol compounds commonly found in food and beverages. Some of the food and beverage products sold in the market contain ethanol. In accordance with that, the objective of this research was to analyse the ethanol content of kombucha tea which is stored at a certain duration and temperature. The research experiment used commercial and homemade kombucha teas. The test was performed at 2 contact temperatures, 8°C (chiller) and 28°C (room temperature) for a duration of 4 weeks. Each sample was analysed for ethanol content, every 1 week using a gas chromatograph coupled with a single quadrupole mass spectrometer (GC-FID). The results demonstrate that storage temperature significantly influences ethanol production, with higher temperatures yielding elevated ethanol content. The ethanol content of commercial kombucha tea stored for 28 days at 28°C was the highest ethanol content (3.43±0.06% ABV). The lowest ethanol content was found in kombucha green tea (1.55±0.11% ABV) which was kept in the chiller (8°C) for the duration of 28 days. It was found that storage temperature and duration play important roles in the concentration of ethanol content. These findings provide producers and regulators with valuable insights for optimising storage practises, promoting halal compliance, and ensuring food safety while preserving fermented beverage's quality and flavour.

Keywords: Ethanol, Temperature, Storage time, Fermentation

1 Introduction

Kombucha tea is a fermented sugared tea that has become popular due to its flavour and potential health benefits. It is produced by fermenting sweetened tea with a symbiotic culture of bacteria and yeast (SCOBY) [1]. In recent years, kombucha has become widely consumed not only in China but also in many other Western countries, Asian countries, the Middle East, and Europe [2-4]. Kombucha tea is considered a functional beverage because of its possible preventative and therapeutic advantages in maintaining health. It was reported that kombucha tea has antioxidant and anti-inflammatory effects due to the presence of polyphenols, which can help protect against chronic diseases such as heart disease and cancer [5-7] Furthermore, the probiotics found in kombucha have been linked to reduced risk of obesity [8,9].

The fermentation process in kombucha involves the conversion of sugars in the tea by yeast and bacteria into various metabolites, including ethanol. Ethanol is a normal by-product during fermentation. This possibility of continued fermentation may lead to an increase in

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ethanol content from levels below 0.5% alcohol by volume (ABV) at time of production to higher levels at time of consumption. Thus, there is a potential for levels to rise to greater than 0.5% ABV, the threshold for certification as a non-alcoholic beverage. Thus, we aimed to assess the presence of ethanol in teas by determining the % ABV of kombucha beverages over time when kept in a refrigerator versus when kept at room temperature.

2 Materials and Methods

2.1 Preparation of Kombucha Teas

Commercial kombucha tea, black tea and green were purchased from a local market near Kuantan, Pahang, Malaysia. Symbiotic Culture of Bacteria and Yeast (SCOBY) was provided by the Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Pahang, Malaysia and it was used as a starter culture of kombucha. Black tea and green tea were fermented using SCOBY following a method from [10] with modification. Boiled water was mixed with 15 g/L of tea leaves. The solution was filtered out, and 11% (w/v) sugar was added to it. The fermentation process was performed in a 500 mL glass jar. The mixture of tea water and sugar was cold down at room temperature. SCOBY was added to the solution. The glass jar was covered by cotton, then kept at room temperature (28 °C) and in the refrigerator (8 °C). The storage times and incubation periods were 5, 6, 7, 14, 21, and 28 days.

2.2 Chemicals and Standard Solutions

All chemicals which are ethanol standard, ethanol absolute (≥99.89%), and propanol that were used throughout the assays were purchased from Sigma-Aldrich-Merck KGaA, Darmstadt, Germany. The ethanol standard solutions were prepared at 5 different concentrations ranging from 0.1 to 1.6% v/v.

2.3 Determination of Ethanol Content

The quantitative analysis of ethanol was performed using a modification method by Mansur et al.[10] 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 were set at 223 ℃ and 250 ℃, respectively and the oven temperature was set initially at 45 ℃ for 1 min, and then increased to the final temperature of 245 ℃ in 1 min at the rate of 10 ℃/min.

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2.4 Data Analysis

The data obtained were analysed using Microsoft Excel 2020 (NY, USA). The results were statistically evaluated using ANOVA (t-test) (p=0.05).

3 Results

3.1 Ethanol Content in Kombucha Teas

As in Table 1, the highest value of ethanol was found in commercial kombucha tea with 3.43±0.06% ABV stored at room temperature for 28 days. Ethanol values of more than 1% ABV were detected in most of the samples of kombucha teas, which exceeded the 1% ABV regulation value. By comparing homemade kombucha tea, kombucha black tea shows high ethanol content compared with kombucha green tea ($p > 0.05$).

3.2 Effect of Storage Temperature and Duration on Ethanol Content

By referring to Figure 1, the ethanol content of kombucha teas tends to increase in all samples over the 28-day period (1.00-3.4% ABV). Kombucha teas stored at room temperature (28 °C) consistently show higher ethanol content compared to refrigerated samples, indicating that temperature plays a significant role in the rate of ethanol production during fermentation. Ethanol content varies based on the type of tea used to make the kombucha, with black tea generally yielding higher ethanol levels compared to green tea. Commercial kombucha demonstrates relatively higher ethanol content compared to homemade kombucha.

Table 1: *Ethanol content (% ABV) at 8 °C and 28 °C for 28 days*

		Ethanol content (% ABV)						
Sample	**Condition**	**Day 1**	**Day 5**	**Day 6**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Commercial kombucha	Refrigerated	1.33±0.08	2.11±0.08	2.31±0.14	2.52±0.10	2.51±0.25	2.54±0.14[a]	2.6±0.14[a]
	Room temperature	1.33±0.06	2.53±0.14	2.73±0.09	2.85±0.07	3.00±0.15	3.24±0.13[a]	3.43±0.06[a]
Kombucha black tea	Refrigerated	0.14±0.02	0.57±0.07	0.9±0.07	1.02±0.12	1.62±0.11	1.72±0.09	1.74±0.10
	Room temperature	0.16±0.02	0.64±0.05	1.13±0.11	1.76±0.08	2.13±0.13	2.65±0.05[a]	2.92±0.06[a]
Kombucha green tea	Refrigerated	0.17±0.01	0.39±0.06	0.49±0.07	0.65±0.04	1.12±0.10	1.45±0.07	1.55±0.11
	Room temperature	0.15±0.01	0.47±0.05	0.56±0.05	0.82±0.02	1.25±0.11	1.57±0.07[a]	1.75±0.10[a]

[a]The mean value within each row shows a significant ($P > 0.05$) difference.

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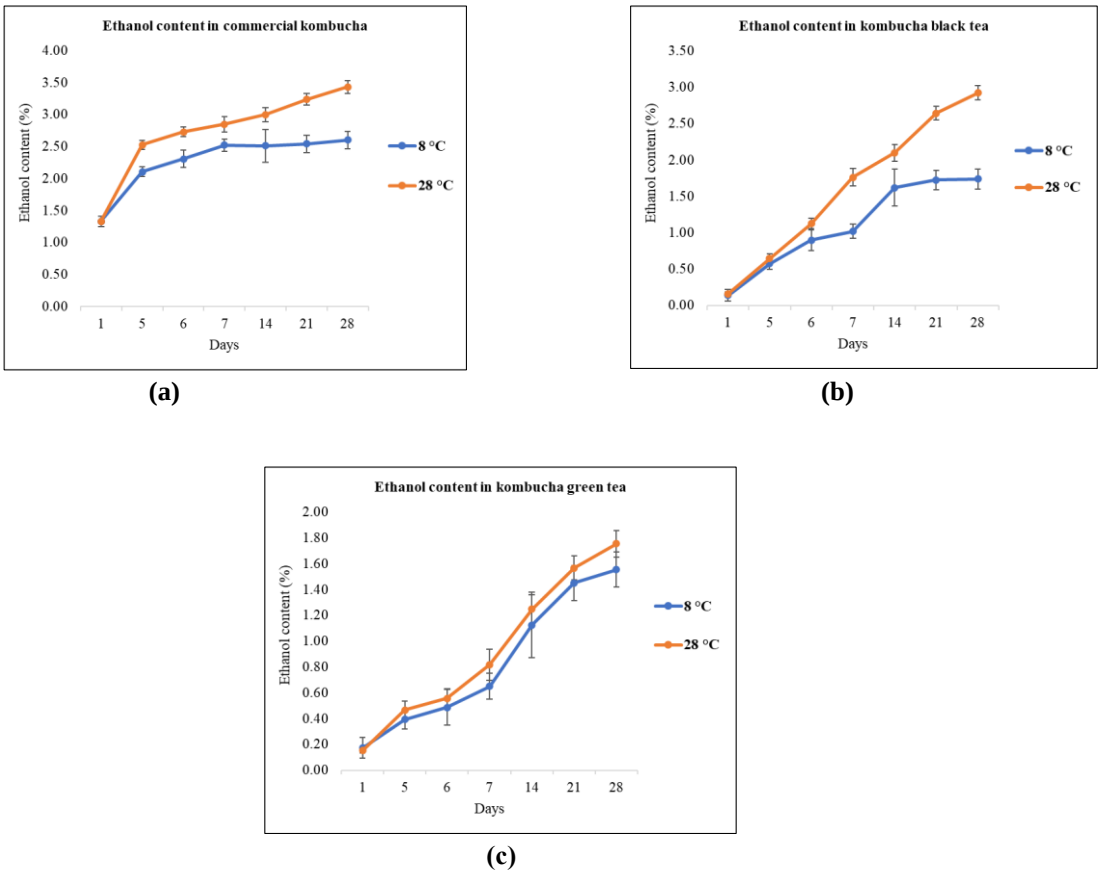

Figure 1: *Ethanol content (% ABV) at 8 °C and 28 °C for 28 days: **(a)** Commercial kombucha tea; **(b)** Kombucha black tea; **(c)** Kombucha green tea.*

4 Discussion

The given data on ethanol content in kombucha teas, with variations in storage duration and temperature, is consistent with and contributes to the larger context of fermented beverage research. Previous research has shown that storage temperature has significant effects on ethanol generation in kombucha. M. et al. [11] proved that higher temperatures accelerate fermentation rates, resulting in more ethanol being produced in a shorter period of time [11]. The greater ethanol level observed at room temperature is consistent with the findings of Taghreed et al. [12], who indicated that elevated temperatures promote sugar metabolism and, as a result, increase alcohol production [12]. This temperature-dependent impact is attributed to yeast strains having increased metabolic activity in warmer settings, resulting in greater sugar conversion to ethanol.

5 Conclusions

The data demonstrates the effect of tea type and storage temperature on ethanol production in kombucha. The observed increase in ethanol content over time suggests that prolonged storage may result in higher ethanol levels, which may have implications for flavor, beverage quality, halal and food safety regulation. To avoid the health concerns associated with excessive alcohol intake, ethanol, a natural byproduct of fermentation, must be kept below

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acceptable levels. Proper ethanol concentration monitoring is consistent with broader food safety guidelines, which aim to safeguard consumers from potential dangers. Overall, this research provides actionable insights for both manufacturers and regulators to ensure that kombucha products follow halal criteria and food safety laws. Producers can develop techniques to maintain safe and compliant alcohol content by knowing how storage conditions and fermented beverages influence ethanol levels.

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