Exploring Kombucha Growth: pH, Sweeteners, and Bacterial Dynamics

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Abstract

Kombucha is widely known to have many health benefits, primarily due to its acidic nutritional content assisting in digestive processes[3]. The kombucha culture (SCOBY) is composed of yeast and bacteria that ferment by feeding on available sugars[1]. This experiment investigated how the pH and bacterial content of a SCOBY changed when fermented in environments with various sweeteners. We hypothesized that bacterial growth and pH would vary between sweetener options. Our hypothesis was supported by measured changes in pH level and bacterial growth between samples, but many samples did not exhibit any bacterial growth after plating.

Introduction

Kombucha, a probiotic-rich beverage, is created by fermenting a sugary tea with a Symbiotic Culture of Bacteria and Yeast (SCOBY). Common bacteria in SCOBYs are Lactobacillus, Gluconacetobacter, and Acetobacter[5]. Yeasts break down sucrose from sweeteners like cane sugar into glucose, fructose, and ethanol. Bacteria then use ethanol and glucose to produce acetic and gluconic acid[4]. As SCOBY, tea, and sugar ferment, pH decreases due to acid production, and alcohol content increases from yeast-synthesized ethanol.

This study explores the impact of sweeteners (honey, stevia, pineapple juice, maple syrup, and agave) on fully-fermented kombucha, comparing pH, acetic acid, ethanol content, and biofilm development in various samples. We expect to see greatest levels of fermentation and bacterial diversity in the sucrose sample as sucrose is the most commonly used sweetener.

Methods

Materials & Location

SCOBY (prepared by WCC collaborators), Loose Leaf Black Tea, Natural Agave, Organic Honey, Natural Maple Syrup, Pineapple Juice, Pure Stevia, Sucrose, Thermo Scientific Elite pH Pocket Tester, Microwave, Analytical Balance; Whatcom Community College Kulshan Hall Science Lab 108; Winter Quarter 2024.

Procedure

Added 25 mL SCOBY to (7) 200-mL bottles. Prepared black tea into (7) 200 mL portions, sweetened with 22.5 g of Agave, Honey, Maple Syrup, Pineapple Juice, Stevia, Sucrose (positive control), and no additives (negative control[2]. After 12-day fermentation, weighed bacterial cellulose to determine yield, and recorded pH.

Figure 1. Experiment Apparatus: Kombuchas with various added sweeteners fermenting. Left to right: Agave, Honey, Maple Syrup, Pineapple Juice, Stevia, Sucrose (Positive Control), and no additives (Negative Control).

Samples that Exhibited Bacterial Growth

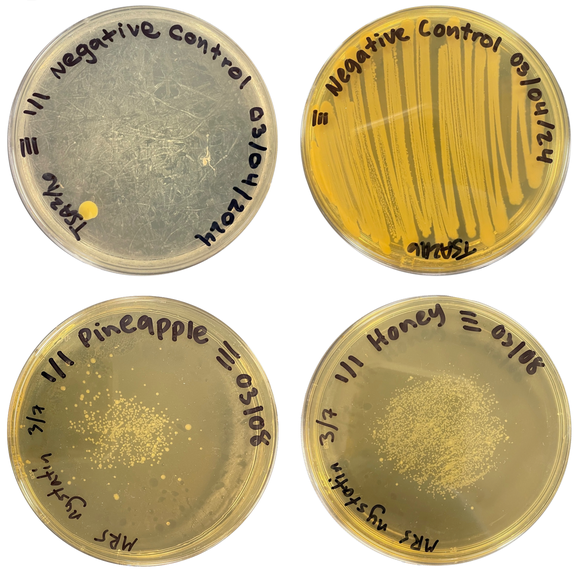

Figure 2. Initial growth of Sporosarcina sp in the negative control at week one (top left) replated (top right). Further growth of a different colony observed during week four but unidentified due to time constraints (bottom).

Sweetener's Effect on pH and Cellulose Yield

Added Sweetener	pH after Fermentation	Bacterial Cellulose Yield
Agave	3.70	29%
Honey	3.60	63%
Maple Syrup	4.47	25%
Pineapple Juice	4.14	36%
Stevia	4.30	15%
Sucrose (Positive Control)	3.90	25%
No Additives (Negative Control)	4.09	N/A

Table 1. pH and bacteria cellulose yield of kombuchas with various sweeteners after fermentation. Honey exhibited the lowest pH and the highest yield.

Discussion

This study set out to determine the magnitude of influence that the type of sweetener used had on the overall composition of the kombucha culture. Samples containing different sweeteners had changes in both pH and percent yield. The honey sample had both the lowest pH (3.60) and the highest percent yield (63%) while both the maple syrup and stevia had higher pHs (4.47 and 4.30 respectively) and lower yields (25.38% and 15.05%). As previously mentioned, the pH can be used as an indicator of the level of fermentation with a lower pH indicating higher levels of fermentation. Both acetic and gluconic acids are synthesized by bacteria from the broken-down sugars provided by the yeasts which means, in order for the acids to be produced, the sugars from the sweetener added have to be broken down[4].

While values for pH and biofilm yield were able to be established, bacterial diversity was not able to be studied to completion. After repeatedly plating both diluted and undiluted samples on both TSA and MRS plates with and without nystatin, one colony was developed after the one-week mark. After 12 days, there was growth on both the pineapple and honey sample plates, but we were unable to pursue sequencing. The initial bacteria was identified as *Sporosarcina sp.* Seeing as this genus is not commonly reported in kombucha samples, there is reason to question the presence of bacteria in the SCOBY kit or suggest that the bacteria present have a longer gestational period than the time allotted. For future research, allowing more time for bacterial growth could yield more conclusive results on bacterial diversity.

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