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Analysis of Microbial Dynamics and Physiochemical Characteristics of Novel Sorghum Fermented Beverages

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Contents

List of Tables 5
List of figures 6
List of Appendices 7
Abstract 8
Resumen 9
Introduction 10
Materials and Methods 13
Study location 13
Materials 13
Methods 13
Sorghum Kombucha Preparation 13
Sampling Plan 13
Physicochemical Measures 13
Microbiological Analysis 13
Total Phenolics Content 14
Experimental Design and Statistical Analysis 14
Results and Discussion 15
Results 15
Microbial Growth Dynamics 15
Qualitative Changes 17
Physiochemical Characteristics 18
Discussion 20
Conclusions 22
Recommendations 23
References 24

Appendices..27

List of Tables

Table 1 Microbiological Analysis performed on days 0, 3, 5 and 7. 14

Table 2 pH changes in Kombucha brewed with sorghum extracts during the fermentation process. 18

Table 3 °Brix changes in Kombucha brewed from sorghum extract throughout the fermentation process. 19

Table 4 Mean ± standard deviation for total polyphenol content in Kombucha brewed with sorghum extract during the fermentation process. 19

List of Figures

Figure 1 Microbial growth in kombucha brewed from Sumac sorghum extract. 15

Figure 2 Microbial growth dynamics in Waxy sorghum Kombucha 16

Figure 3 Microbial growth in kombucha brewed from White Sorghum extract. 17

Figure 4 Sorghum Kombucha treatments at day 7. 18

List of Appendices

Appendix A Microbial growth curve in Sumac Sorghum Kombucha 27

Appendix B Microbial growth curve in Waxy sorghum Kombucha 28

Appendix C Microbial growth curve in White Sorghum Kombucha 29

Abstract

Kombucha is a fermented drink of Asian origin that has been attributed with multiple health benefits and functional properties. Traditionally, this beverage is made from sweetened black or green tea and a starter culture (SCOBY). This study aims to explore the microbial dynamics and physiochemical changes during kombucha fermentation using three types of whole grain sorghum: White, Waxy, and Sumac. During fermentation, pH, °Brix, and polyphenol content were evaluated as well as the growth dynamics of lactic acid bacteria, acetic acid bacteria, and yeast. It was found that the tannin content of the sorghum varieties affected microbial growth, specifically acetic acid bacteria. In addition, there were significant differences in the polyphenol content between treatments, with Sumac sorghum kombucha exhibiting the highest polyphenol content on day seven. These findings suggest that the presence of tannins in sorghum may impact bacterial growth during Kombucha fermentation. Therefore, it is recommended to use sorghum high in tannins, such as Sumac Sorghum, for kombucha fermentation. However, further research is needed to identify the specific microbial interactions and determine the precise mechanism underlying the influence of sorghum tannins on bacterial growth.

Keywords: Kombucha, microbial dynamics, phenolic content, sorghum.

Resumen

La Kombucha es una bebida fermentada de origen asiático a la cual, se le han atribuido múltiples beneficios para la salud y propiedades funcionales. Tradicionalmente, esta bebida es elaborada a partir de té negro o verde endulzado y un cultivo iniciador (SCOBY). Este estudio tiene como objetivo explorar la dinámica microbiana y los cambios fisicoquímicos durante la fermentación de Kombucha utilizando tres tipos de sorgo integral: Blanco, Ceroso y Sumac. Durante la fermentación, los parámetros pH, grados Brix y el contenido de polifenoles fueron evaluados, así como la dinámica de crecimiento de las bacterias ácido-lácticas, ácido-acéticas y levaduras. Los resultados observados indicaron que el contenido de taninos presente en las variedades de sorgo influenció las dinámicas de crecimiento microbiano, específicamente el de las bacterias ácido-acéticas y ácido-lácticas. Así mismo, diferencias significativas fueron observadas en el contenido de polifenoles entre los tratamientos durante la fermentación, siendo la Kombucha de sorgo Sumac la que presentó el mayor contenido de polifenoles al finalizar el proceso fermentativo. Los hallazgos de este estudio sugieren que la presencia de taninos influye. Estos hallazgos sugieren que la presencia de taninos en el sorgo puede afectar las dinámicas de crecimiento microbiano durante la fermentación de Kombucha. Por lo tanto, se recomienda utilizar sorgo alto en taninos, como el sorgo Sumac, para la fermentación de la Kombucha. Sin embargo, es necesario mayor investigación para lograr identificar las interacciones microbianas específicas y determinar el mecanismo preciso que subyace en la influencia de los taninos del sorgo en el crecimiento bacteriano.

Palabras clave: Contenido de polifenoles, dinámicas microbianas, kombucha, sorgo.

Introduction

In recent years, there has been a growing interest in functional foods, as individuals become more health-conscious, they actively seek out products that not only fulfill their dietary requirements but also contribute to their overall well-being.

Kombucha is a fermented drink of Asian, its consumption dates to around 220 BC in the Tsin Dynasty in China, where it was referred to as the "Divine Che" for its detoxifying and energizing properties (Greenwalt et al., 2000). This beverage has gained popularity in the west, due to its multiple functional properties such as anti-inflammatory potential, antioxidant, and antimicrobial activity (Chakravorty et al., 2016; Villarreal-Soto et al., 2018; Watawana et al., 2015). Kombucha is also associated with influencing the gastrointestinal microbial flora in humans by acting as a probiotic drink and help to balance the intestinal flora (Malbaša et al. 2011; Jayabalan et al. 2014; Dufresne and Farnworth 2000).

The traditional drink is a product fermented from sweet or black tea by a symbiotic culture of bacteria and yeast (SCOBY). It contains unique combinations of bacteria and yeast, but commonly found strains consist of acetic acid bacteria (AAB), such as *Komagataeibacter*, *Gluconobacter*, and *Acetobacter* species (Roos y Vuyst, 2018); lactic acid bacteria (LAB) including *Lactobacillus* sp., *Lactococcus* sp, *Lecunostoc* sp (Chakravorty et al., 2016); and yeasts such as *Schizosaccharomyces pombe*, *Saccharomycodesc ludwigii*, *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* (Antolak et al., 2021; Coton et al., 2017).

There are several types of fermentation and products obtained depending on the metabolic pathway followed. Kombucha fermentations is a combination of three of them: alcoholic, lactic, and acetic. During the primary fermentation stage, yeast cells from the initial inoculum hydrolyze sucrose into glucose and fructose using invertase or sucrase enzymes. The fructose released through invertase action is mostly consumed by yeast cells via glycolysis, resulting in the conversion of fructose into ethanol and carbon dioxide. Acetic acid bacteria rapidly oxidize the produced ethanol into acetic acid and oxidize glucose into gluconic, acid which is further converted into glucuronic acid, which has been recognized for its hepatoprotectives effects. Additionally, under the influence of lactic acid bacteria, glucose is converted into lactic acid (Jakubczyk et al., 2022; Jayabalan et al., 2017; Martínez-Leal et al., 2020; Nguyen et al., 2015).

In recent years, new fermentation substrates have been studied in order to find new bioactive compounds that permit additional health claims, as well as to improve or diversify the sensory properties of Kombucha (Liu et al., 2022). In a study conducted by Ayed et al. (2017) the use of grape juice as an alternative substrate was evaluated resulting in a notable augmentation of phenolic content. Likewise, Sun et al. (2015) observed a substantial increase in diverse polyphenols when blending wheatgrass juice with traditional kombucha. Similarly encouraging outcomes have been observed in similar studies, wherein the utilization of alternative substrates such as pomegranate juice, sour cherry juice, apple juice, and black carrots showcased improvements in key sensory aspects, including color and flavor (Akbarirad et al., 2017; Yilds et al., 2021).

One such ingredient that holds promise is sorghum, a versatile grain that has been recognized for its nutritional value and adaptability to various environmental conditions. Sorghum (*Sorghum bicolor* (L.) Moench) is a crop that is widely grown all over the globe for food and feed purposes. The United States is the world's largest sorghum producer, accounting for approximately 24% of global sorghum production (Ciampitti et al., 2019). The majority of the US sorghum production, approximately 90%, is concentrated in the states of Kansas, Texas, South Dakota, Colorado, and Nebraska (USDA, 2022). This gluten-free grain is a rich source of nutrients and contains a diverse range of bioactive phenolic compounds (Dykes y Rooney, 2006). Sorghum can be classified into two major types based on the presence or absence of tannins, according to the literature there is a link between tannin concentration and anti-inflammatory potential (salman et al., 2021). Studies suggest that the tannin-rich sorghum extracts may have an anti-inflammatory and disease-associated effect (Hong et al., 2020; Meena et al., 2022). Selecting varieties of sorghum with high concentrations of bioactive compounds could be an effective strategy to confer benefits to human health.

Considering the growing interest in functional foods, the established importance of kombucha as a probiotic beverage, as well as the multiple attributes and benefits of sorghum, evaluating the incorporation of this grain as an ingredient in kombucha fermentation holds immense potential. Therefore, this study aims to explore the microbial dynamics and physicochemical changes during kombucha production fermented from alternatives substrates, three types of whole grain sorghum: White, Sumac, and Waxy.

Materials and Methods

Study Location

The study was conducted in the Department of Animal Science and Industry at Kansas State University, the kombucha fermentation process and the analyses performed were carried out in the call hall 202 Food Microbiology and Safety Laboratory.

Materials

Three types of whole sorghum grains, White (NLSC-WSWG-0010), Red (Waxy Burgundy, NLSC-WBSWG-0014L), Sumac (NLSC-SUSWG-0018), provided by Nu Life Market, KS, and an Organic Kombucha Scoby-Live Culture, bought on Amazon, were used in this study.

Methods

Sorghum Kombucha Preparation

A grain-to-water- ratio of 1:3 grain- water was employed, where 230 grams of whole grain sorghum were rinsed three times until water ran clear, then added to 690 mL of Deionized (D.I.) water and left overnight at room temperature conditions. Two cups of the overnight extract were strained and boiled. Then it was added to a sanitized gallon glass jar and mixed with 1 cup of pure granulate cane sugar and 8 (1:8 ratio) cups of Deionized (D.I.) water. Once the mixture temperature was below 35 °C, the starter culture SCOBY and the starter tea were introduced into the mixture. The jar was covered with coffee filters and incubated at 27 °C.

Sampling Plan

On days 0, 3, 5 and 7, 5 mL aliquots from each Kombucha were taken by using a sterile pipette.

Physicochemical Measures

On days 0, 3, 5, and 7 an aliquot of each sample was taken for the measurements of pH and °Brix. The pH was measured with a handheld pH meter VWR Traceable®Digital pH/ORP and °Brix was measured with a handheld portable refractometer (Extech Brix Refractometer).

Microbiological Analysis

On days 0, 3, 5 and 7 acetic acid-producing bacteria and lactic acid bacteria growth, were monitored by plating using the pour plate technique on Wallerstein Laboratory nutrient WL agar (HiMidia, Mumbai, India) and Man-Rogose-Sharpe MRS agar (Difco, Detroit, USA). The plates were incubated at 27 °C for 48 hours and 35 °C for 72 hours, respectively. For the enumeration of yeast and molds, 1 mL of the liquid samples were plated on Petrifilm™ Yeast and Mold Count Plate (3M™, Saint Paul, Minnesota, USA) and incubated at 27 °C for up to five days. A duplicate of each sample, serial dilution, and plating was duplicate (Table 1).

Table 1

Microbiological Analysis performed on days 0, 3, 5 and 7.

Microorganisms	Medium	Incubation time
Acetic acid bacteria	Wallerstein Laboratory nutrient WL agar (HiMidia, Mumbai, India)	27 °C/ 2 days
Lactic acid bacteria	Man-Rogose-Sharpe MRS agar (Difco, Detroit, USA)	37 °C/ 3 days
Yeast and mold	Petrifilm™ Yeast and Mold Count Plate (3M™, Minnesota, USA)	27 °C/ 5 days

Total Phenolics Content

The amount of total phenolics content in kombucha was determined with the Folin- Ciocalteu reagent. Gallic acid was used as a standard and total phenolics were expressed as mg/g gallic acid.

Experimental Design and Statistical Analysis

Completely Randomized Design with repeated measurements over time was used in this study. The independent variables were whole grain sorghum variants (White, Waxy, and Sumac). Three replicates were performed for each treatment, with four sampling time points (days 0, 3, 5 and 7) for nine experimental and 36 observational units respectively. A GLM procedure and Least squares means (LSMEANS) $p \leq 0.05$ was used to evaluate statistical differences and interactions between treatments and times measured.

Results and Discussion

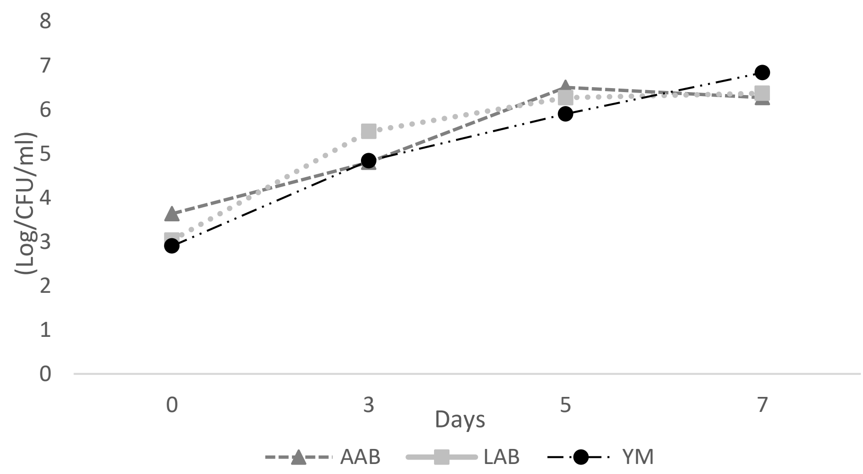
Results

Microbial Growth Dynamics

The microbial growth dynamics in kombucha brewed from Sumac sorghum extract are shown in Figure 1. No significant differences ($p \leq 0.05$.) were found among the population means of the three microorganisms during the fermentation process (counts on days 0, 3, 5, and 7). An upward trend was observed, starting with an average of 3.6 - 2.9 log CFU/mL on day 0 and reaching 6.8 - 6.3 log CFU/mL on day 7. Regarding the growth dynamics, Appendix A, shows variations in the duration of the microbial growth phases among microorganisms, specifically in the yeast population. Yeast exhibited an exponential growth phase from day 0 to day 7, while the exponential growth phase of lactic acid and acetic acid bacteria ended on the fifth day, after which their stationary phase began.

Figure 1

Microbial growth in kombucha brewed from Sumac sorghum extract.



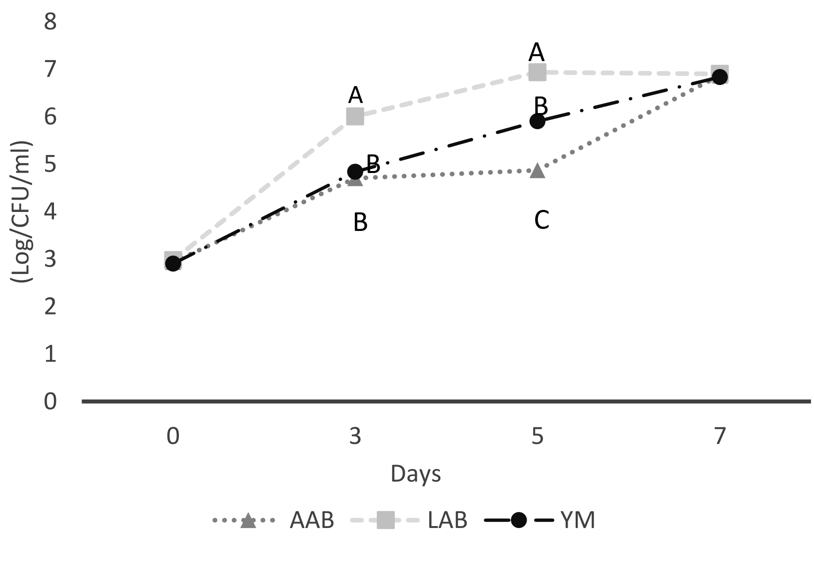
Note. (AAB) Acetic acid bacteria; (LAB) Lactic acid bacteria; (YM) Yeasts.

Figure 2 shows the microbial growth in Waxy sorghum kombucha. Significant differences were observed in the population of the three microorganisms monitored during the fermentation process. However, those differences were only observed on days 3 and 5. On the third day, the lactic acid bacteria population where different from the acetic acid bacteria and yeast population, it exhibited the highest population, with a mean of 6 Log CFU/ml, whereas acetic acid bacteria and yeast had a mean of 4.8 Log CFU/ml. On the fifth day, the three microorganisms' population were significantly different, lactic acid bacteria showed the highest population with a mean of 6.9 Log CFU/ml, followed by the yeast population with a mean of 5.8 Log CFU/ml. Acetic acid bacteria showcase the lowest population with a mean of 4.8 Log CFU/ml. By day 7, no differences were observed among the three microorganisms, the population mean was around 6.8 Log CFU/ml.

In relation to the duration of growth phases, the three microorganisms exhibited distinct behaviors (Appendix B). Yeasts and acetic acid bacteria underwent an exponential phase from day 0 to day 7. However, acetic acid bacteria showed a slower increase in population compared to yeasts, particularly during days 3 to 5. Conversely, lactic acid bacteria were the only ones to enter a stationary phase from day 5 to 7.

Figure 2

Microbial growth dynamics in Waxy sorghum Kombucha

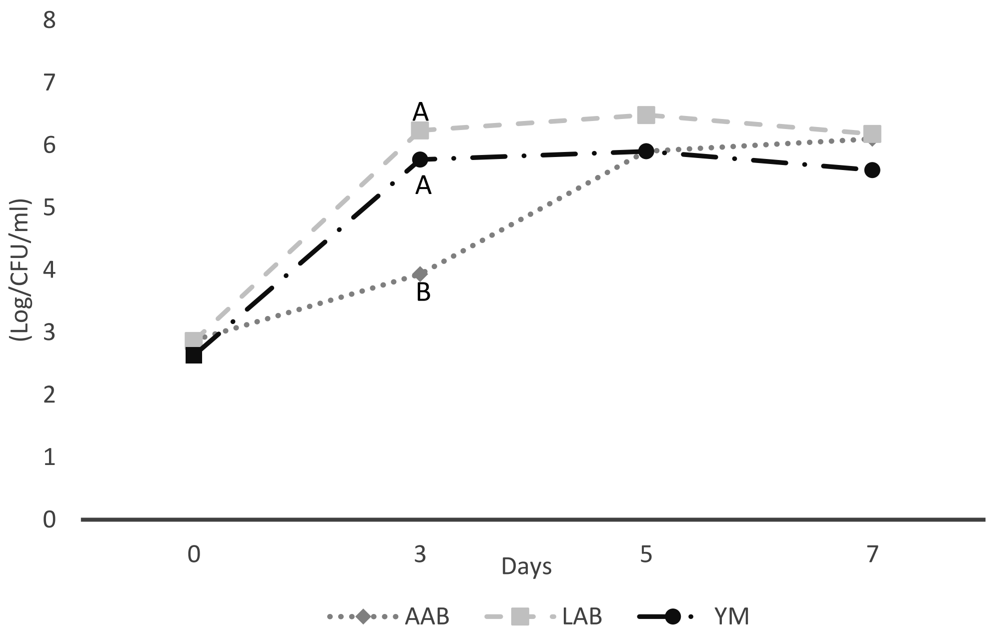


*Note***.** The mean population among microorganisms with different letters (A-C) is significantly different (P≤0.05). (AAB) Acetic acid bacteria; (LAB) Lactic acid bacteria; (YM) Yeasts.

In Kombucha brewed from White sorghum extract (Figure 3), significant differences in microbial growth were observed. However, these differences were only evident on day 3, acetic acid bacteria was the lowest with a mean of 3.9 Log CFU/ml. Differences were also observed in the duration of the microbial growth phases (Appendix B). The exponential growth phase of lactic acid bacteria and yeasts lasted from day 0 to day 3, followed by a stationary phase from day 3 to day 7, while, acetic acid bacteria had a longer exponential phase, from day 0 to day 5, after which, they entered their stationary phase. By day 7 the three microbial populations reached a state of equilibrium.

Figure 3

Microbial growth in kombucha brewed from White Sorghum extract.



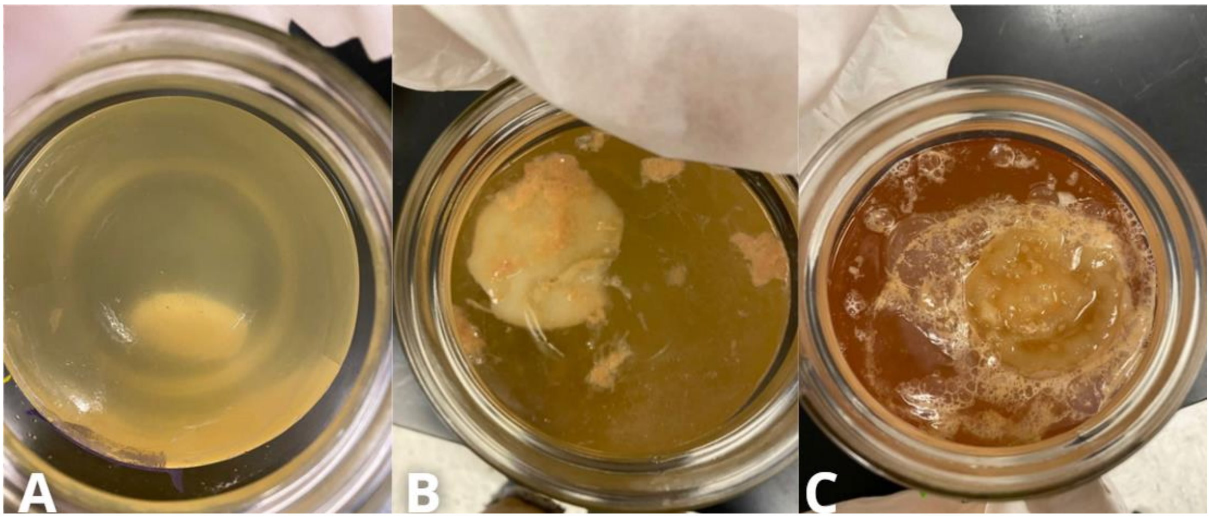
Note. The mean population among microorganisms with different letters (A-B) is significantly different (P≤0.05). (AAB) Acetic acid bacteria; (LAB) Lactic acid bacteria; (YM) Yeasts.

Qualitative Changes

Daily monitoring was conducted to observe the changes in kombucha qualitative characteristics, including color, odor, and the presence of cellulose biofilm (Figure 4). Apart from color, the only noticeable visual difference was the formation of cellulose biofilm on the tea surface. By day 7, Sumac (C), and Waxy (B) showed a well-formed cellulose biofilm, while White kombucha (A) displayed a thinner cellulose biofilm.

Figure 4

Sorghum Kombucha treatments at day 7.



Note. (A) White Sorghum Kombucha (B) Waxy Sorghum Kombucha (C) Sumac Sorghum Kombucha.

Physiochemical Characteristics

The pH data were measured on days 0, 3, 5, and 7 (Table 2). Based on the statistical analysis performed, none of the treatments showed statistically significant differences in pH data. The initial pH values ranged from 3.5 to 3.2 and decreased to 3.0 to 2.9 across all treatments.

Table 2

pH changes in Kombucha brewed with sorghum extracts during the fermentation process.

GRAIN	Days (Mean ± SD)			
	0	3	5	7
Sumac	3.42 ± 0.11	3.27 ± 0.07	3.1 ± 0.03	3.04 ± 0.01
Waxy	3.51 ± 0.04	3.7 ± 0.85	3.16 ± 0.07	3.07 ± 0.09
White	3.27 ± 0.01	3.15 ± 0.05	3.02 ± 0.09	2.9 ± 0.13
C.V. (%)				6.2

Note. SD: Standard deviation; CV: Coefficient of variance (%). No significant differences were observed (P>0.05).

No statistically significant differences were found between treatments in terms of °Brix during the fermentation process. The initial °Brix values were 8 and decreased to 7.5-7.7 by day 7 (Table 3).

Table 3

°Brix changes in Kombucha brewed from sorghum extract throughout the fermentation process.

GRAIN	Days (Mean ± SD)			
	0	3	5	7
Sumac	8.0± 0.10	7.9 ± 0.10	7.8 ± 0.05	7.5 ± 0
Waxy	8.03 ± 0.05	7.9 ± 0.05	7.8 ± 0.1	7.5 ± 0.35
White	8.0 ± 0	7.9 ± 0.05	7.8 ± 0.05	7.7 ± 0.28
C.V (%)				1.8

Note. CV: Coefficient of variance (%). SD: Standard deviation. No significant differences were observed (P>0.05).

The polyphenol content in kombucha brewed from sorghum extract was monitored on days 0, 1, 5, and 7, and reported as mg of gallic acid/L (Table 4). Kombucha brewed from Sumac sorghum extract showed the highest polyphenol content throughout the fermentation process. Its trend was ascending, starting with an average of 111.64 mg gallic acid/L on day 0 and reaching 127.50 mg gallic acid/L on day 7. Regarding White and Waxy kombucha, significant differences were observed between them on day 0, with Waxy showing a higher polyphenol content with a mean of 41.89 mg gallic acid/L, while White sorghum showed an average of 29.72 mg gallic acid/L. However, these differences were only observed on day 0, as no statistically significant differences were found on days 1, 5, and 7.

Table 4

Mean ± standard deviation for total polyphenol content in Kombucha brewed with sorghum extract during the fermentation process.

GRAIN	Days mg gallic acid/L			
	0	1	5	7
Sumac	111.46 ± 3.01 [a]	112.37 ± 3.90[a]	122.77 ± 8.77 [a]	127.50 ± 5.71[a]
Waxy	41.89 ± 0 [b]	42.71 ± 1.49[b]	40.25 ± 3.80[b]	41.75 ± 4.97[b]
White	29.72 ± 0.43 [c]	32.77 ± 1.41 [b]	35.94 ± 1.23 [b]	36.43± 0.56[b]
C.V (%)				6.1

Note. (a-c) means with different letters among sorghum grains for each day are significantly different (P ≤ 0.05). (CV)Coefficient of variance (%).

Discussion

The Kombucha fermentation process entails competitive and cooperative interactions among microorganisms, resulting in a multitude of biochemical changes, including variations in pH values, °Brix levels, and microbial growth, among others. Kombucha brewed from Sumac sorghum extract demonstrates a balanced relationship between microbial populations during fermentation. This initial increase in acetic acid bacteria and yeast, as well as the formation of a cellulose biofilm by the acetic acid bacteria, is consistent with the findings reported by Villarreal-Soto et al. (2018). Likewise, the initial increase in lactic acid bacteria population followed by a gradual decrease aligns with the findings of Ayed et al. (2017).

Conversely, Waxy and White demonstrated distinct microbial growth patterns and biofilm formation. These differences can be influenced by several factors, including the composition of the substrate. According to Wang et al. (2020), the presence of tannins has been shown to significantly enhance fermentation quality and promote the growth of acetic acid bacteria, responsible for cellulose production in kombucha fermentation (Yamada et al., 2012). Throughout the fermentation process, Sumac sorghum kombucha exhibited the highest polyphenol content, while White sorghum kombucha reported the lowest tannin content (Table 4). This may explain why the cellulose biofilm in white sorghum kombucha appeared thinner than that in Waxy and Sumac Kombucha by day 7.

Sorghum grain possesses various phenolic acids and flavonoids, including flavanones, flavanols, anthocyanins, and condensed tannins, also known as proanthocyanidins (Xu et al., 2021). The presence and concentration of these compounds can vary among different sorghum varieties. White sorghum and Waxy sorghum can be classified as tannin-free sorghums, whereas Sumac is classified as tannin sorghum (Awika et al., 2009). Hence, Sumac kombucha exhibits the highest polyphenol content among the treatments by the end of the fermentation. These variations in the polyphenol content may elucidate the differences observed in the growth dynamics of acetic acid bacteria and, consequently, the formation of cellulose biofilm in each treatment.

According to the literature, the presence of some polyphenols can have a negative effect on certain strains of lactic acid bacteria, causing a delay in their glucose metabolism (Campos et al., 2009; Piekarska-Radzik y Klewicka, 2021). Given that glucose serves as an energy source for both lactic acid and acetic acid bacteria, it is plausible that the lower presence of polyphenols observed in Waxy and White sorghum kombucha promotes the growth of lactic acid bacteria, thereby increasing competition for glucose with acetic acid bacteria. However, further investigation is warranted to identify the specific species involved and their respective responses and preferences in environments with varying tannin concentrations in kombucha fermented from sorghum extracts.

Moreover, the °Brix and pH results obtained in the three treatments align with previous literature findings. The observed decreasing trend in °Brix can be attributed to the breakdown of sucrose into glucose and fructose by yeast invertase enzymes, as reported by Oliveira et al. (2022). Additionally, the decline in pH values can be ascribed to the production of diverse organic acids, such as acetic acid, lactic acid, and glucuronic acid, by the symbiotic culture of bacteria and yeast (Jayabalan et al., 2010).

Overall, these findings highlight the intricate dynamics and interactions within the kombucha fermentation process, emphasizing the influence of microbial populations, substrate composition, polyphenol content, and biochemical changes.

Conclusions

Sorghum extract can be used as an effective substrate to produce Kombucha with high added value and functional properties. The presence of tannins in sorghum may impact bacteria growth, particularly the growth of acetic acid bacteria. Therefore, it is recommended to use tannin sorghum varieties, such as Sumac Sorghum for kombucha fermentation. However, further research is needed to identify the specific microbial interactions and determine the precise mechanism underlying the influence of sorghum tannins on bacterial growth.

The growth dynamics and microbial populations among kombucha brewed from three types of sorghum extract exhibited differences during the fermentation process. However, by the end of the fermentation process, the three microbial populations in each treatment showed a state of equilibrium where none of them differed statistically from each other. In terms of the physicochemical parameters, the only differences found were in the polyphenol content, where Sumac sorghum kombucha exhibited the highest polyphenol content throughout the fermentation process.

Recommendations

Identify the phenolic compounds present in each type of sorghum extract, as well as the microbial species within the starter culture, to better understand their responses to various concentrations and types of phenolic compounds.

Implement a control treatment to compare and analyze the microbial dynamics and physicochemical changes between traditional kombucha and sorghum kombucha.

Conduct a sensory analysis to evaluate the acceptance of the treatments.

Perform an in vitro study to assess the probiotic potential of the kombucha brewed from sorghum extract.

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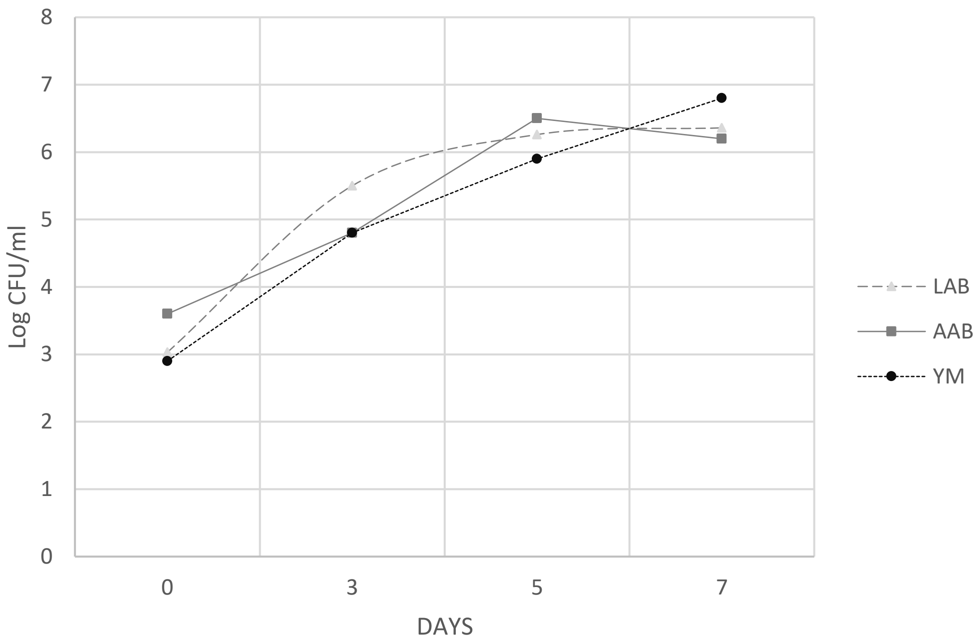
Appendices

Appendix A

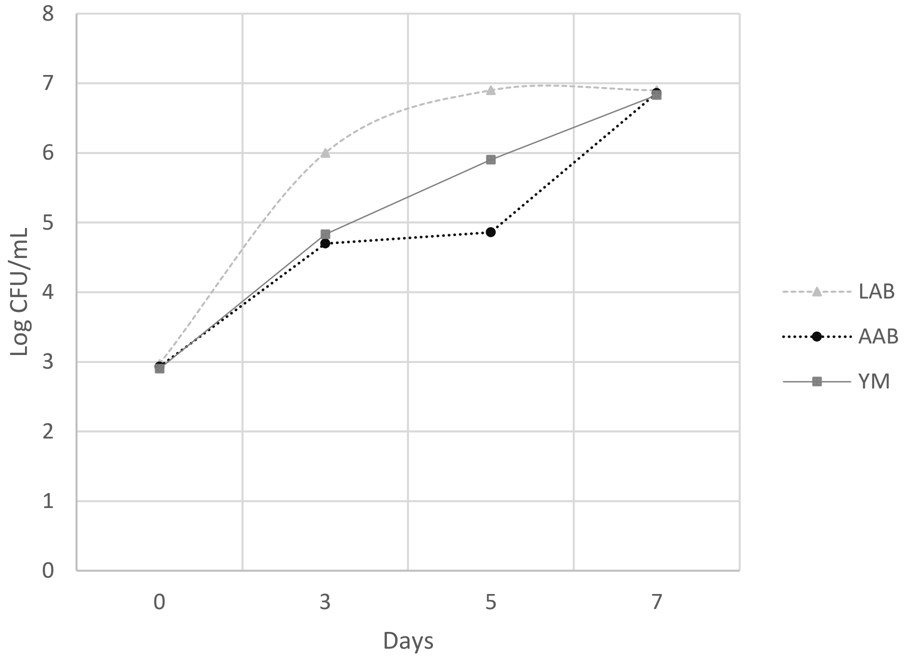
Microbial growth curve in Sumac Sorghum Kombucha



Note. (LAB) Lactic acid bacteria, (AAB) Acetic acid bacteria, (YM) Yeasts.

Appendix B

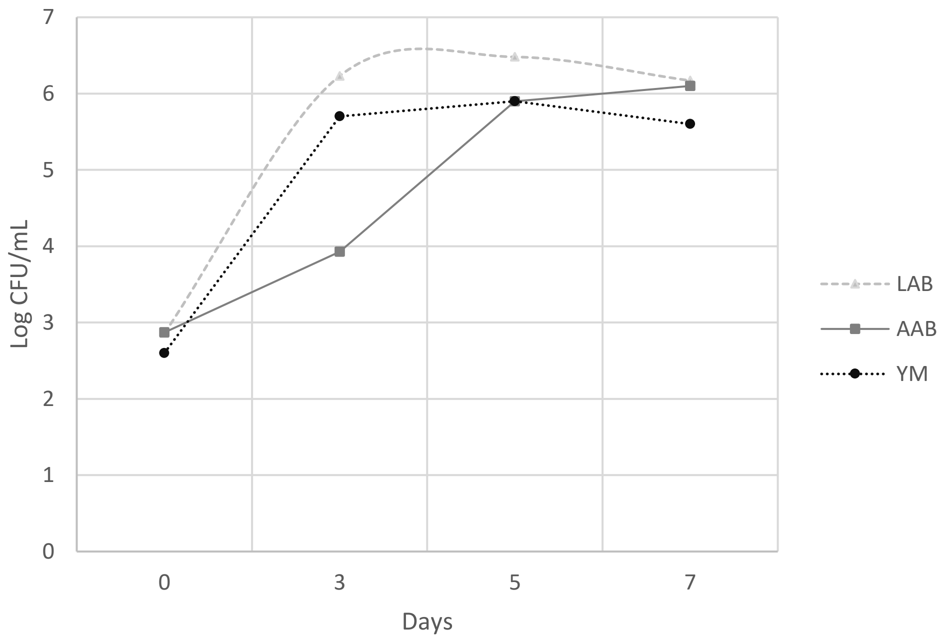
Microbial growth curve in Waxy sorghum Kombucha



Note. (LAB) Lactic acid bacteria, (AAB) Acetic acid bacteria, (YM) Yeasts.

Appendix C

Microbial growth curve in White Sorghum Kombucha



Note. (LAB) Lactic acid bacteria, (AAB) Acetic acid bacteria, (YM) Yeasts.