Evalution of gut microbiota after colonic fermentation in the presence of kombuchas with and without sugar

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ABSTRACT

This study evaluated the effect of added sugar in the fermentative process of kombucha on the human gut microbiota. The beverage without sugar influenced the beneficial microbiota (*Bifidobacterium*-33%), while the sweetened beverage favored the microbiota associated with human diseases such as *Escherichia shigella,* increasing the relative abundance of this genus by 57.8%. Short-chain fatty acids increased in all samples after 48 hours of fermentation.

Keywords: Kombucha.gut microbiota. SCFAs. ***Bifidobacterium***. sugar.

1 INTRODUCTION

The human digestive system comprises a vast population of microorganisms, approximately 40 trillion cells. Changes in the balance of this environment are correlated with human diseases. Therefore, foods that can influence intestinal eubiosis are essential for delaying potential diseases caused by dysbiosis.

Fermented beverages such as kombucha are an important nutritional source. This beverage contains microorganisms with probiotic and functional strains, such as polyphenols. These compounds exert effects like those of prebiotics and are associated with a healthy microbiome. The introduction of polyphenols by the intestinal microbiota is also associated with the production of short-chain fatty acids (SCFAs), such as acetate, propionate, butyrate, and lactate. These compounds are known to confer certain health benefits to the host. Conversely, foods that contain sugar and their excessive consumption are associated with metabolic diseases and the increase of pathogenic microorganisms.

Thus, kombucha offers benefits to the intestinal microbiota through the metabolites produced during the fermentation process. This research evaluated the influence of kombucha without and with added sugar in the fermentation process of the beverage, in the modulation of the human intestinal microbiota, and the production of short-chain fatty acids.

2 MATERIAL & METHODS

Kombuchas were prepared by infusing *Camellia sinensis* leaves (1%) in filtered water at 90°C for 15 min and inoculating them with 10% SCOBY and 10% fermented tea. The beverages were identified as traditional Kombucha without sugar (TKS) and traditional Kombucha with sugar (TKWS—3% sucrose). Fermentation was carried out for 9 days at 28±1ºC.

The INFOGEST 2.0 method was used for the in vitro gastrointestinal digestion of traditional Kombuchas with sugar (TKWS) and without sugar (TKS). The fecal inoculum was prepared with fecal samples collected from four healthy human donors on the day of simulated digestion. The experimental procedure was submitted and approved by the Research Ethics Committee (Federal University of Ceará, CEP/UFC, CAAE 56171022.2.0000.5054). The microbiota analysis was made by high throughput 16S rRNA gene sequencing (300-cycles, 2 × 150 bp) using the MiSeq kit v2 reagent on the Illumina MiSeq platform (Illumina Inc., USA) according to Pereira et al., (2022). Taxonomic affiliation was obtained through the DADA2 and Greengenes databases. The Agilent Technologies Infinity 1260 system was used to identify organic acids (Ferreira Leite et al., 2023).

3 RESULTS & DISCUSSION

The metabolites produced during fermentation for TKS- lactic acid (1.46 g/L), acetic acid (2.32 g/L), gluconic acid (2.17 g/L), isdobutyric acid (0.77g/L). TKWS acid concentrations were 1.44 g/L, 5.51 g/L, 2.75 g/L and 1.35 g/L respectively. Furthermore to acids, total phenolic compounds were quantified before and after colonic fermentation. TKS had 108.12 mg GAE/ L and then 4.08 mg GAE/ L. TKWS had 171.78 mg GAE/ L and then 4.91 mg GAE/ L. The results indicate consumption by the fecal microbiota.

Figure 1 shows the changes in the fecal microbiota fermented with kombuchas with and without sugar and the production of SCFAs. In TKWS *Escherichia shigel* (57.8%),*Terrisporobacter* (12.3%), *Eubacterium* (6.03%), *Enterococcus* (4.39%) had the highest abundance after 48 h. The presence of *Escherichia shige*l in TKWS is strongly associated with poor eating habits and metabolic diseases. The genus *Bifidobacterium* was present in the fecal inoculum of all samples; however, after 48h in the presence of TKWS, there was no significant difference in abundance. In TKS, there was an increase of 33,8% in relative abundance. This species is among the most abundant in the human large intestine, and its abundance can be affected by diets high in low molecular weight carbohydrates. Non-digestible compounds such as prebiotics stimulate an increase in the abundance of Bifidobacterium. A greater production of SCFAs was observed in TKS than in TKWS after 48 hours of fecal fermentation. The production of these metabolites is related to the metabolism of *Bifidobacteria, Lactobacillus, Bacteroides*, and *Clostridium*. Butyrate and propionate had higher concentrations in TKS (38 g/L and 13g/L, respectively) than in TKWS (10,13g/L and 9,73%) after 48h and fermentation colonic. Butyrate stabilizes the pH of the intestinal lumen, improving nutrient absorption, and propionate acts in the regulation of metabolism via immune function, lipid metabolism, and neurotransmitter synthesis.

The interaction between diet and the human intestinal microbiota, especially potentially functional foods such as kombucha, they are associated with the production of metabolites such as SCFAs and modulation of the microbial environment. This observed relationship promotes benefits to human health

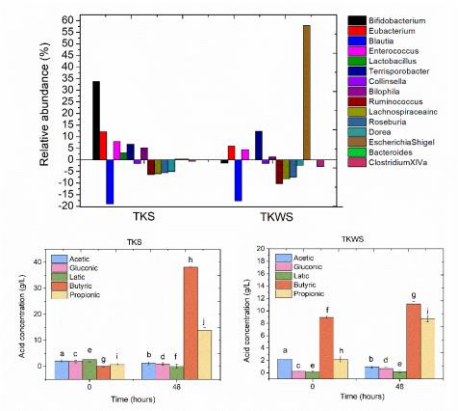

Figure 1. (a) Relative abundance (%) at gender level of fecal microbiota fermented in the presence of TKS and TKWS. Values above 5% were considered significant. Positive values indicate an increase in relative abundance while negative values indicate reduction. (b) concentration of acids during colonic fermentation. The results were expressed as means with standard deviation (n=3). Different letters denote statistical significance (one-way ANOVA, Tukey test, $p \leq 0.05$).

4 CONCLUSION

The digested kombuchas influenced the enrichment of microbiota, especially TKS, favoring the beneficial microbiota. The available reducing sugars for use by microbiota favored the growth of several genera of microorganisms, including those associated with diseases and metabolic disorders. Overall, this study provided important information about the influence of sugar added to kombucha during enteric fermentation. Though this beverage is considered functional, the free sugar concentration can influence the relative abundance of disease-associated microorganisms.

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