

# SIMPÓSIO BRASILEIRO DE MICROBIOLOGIA APLICADA

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## SIMPÓSIO BRASILEIRO DE MICROBIOLOGIA APLICADA

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#### THE INFLUENCES OF MICROBIAL STARTER CULTURES AND SUBSTRATES ON THE CHEMICAL COMPOSITION OF KOMBUCHA

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Kombucha is a fermented Camellia sinensis infusion performed by a complex symbiotic culture of microorganisms. The microbial and biochemical composition of kombuchas vary a lot since products of spontaneous fermentation result in highly variable processes. In this study, we evaluated the potential of Acetobacter aceti (AA), Lactobacillus plantarum (LP), and Kluveromyces marxianus fragilis (KMF) as a selected starter cultures to produce kombucha. Kombucha was produced using 8 g.L<sup>-1</sup> of organic green tea and 60 g.L<sup>-1</sup> of sugar at 28 °C for 10 days. Organic acids (acetic, lactic, and succinic), alcohols (ethanol and glycerol), and residual sugars (sucrose, glucose, and fructose) were analysed by HPLC, and enumeration of microorganisms was performed using selective media. We studied the impact of microbial interactions and sugar type on the chemical composition by running three experiments: 1) inocula of 1.10<sup>7</sup> UFC.mL<sup>-1</sup> of each microorganism using demerara sugar; 2) Identical to experiment 1, using white sugar; 3) Inocula of 1.10<sup>7</sup> UFC.mL<sup>-1</sup> of AA, 1.10<sup>7</sup> UFC.mL<sup>-1</sup> of LP, and 1.10<sup>5</sup> UFC.mL<sup>-1</sup> of KMF, using demerara sugar. All treatments resulted in kombuchas with low amounts of acetic acid (0.4 to 0.5 g.L<sup>-1</sup>) and similar succinic acid concentrations (0.17 to 0.19 g.L<sup>-1</sup>). The initial cell amount of KMF in treatments 1 and 2 strongly impacted the viability of LP and AA cells, the yeast being the only viable microorganism after 10 days. Sugar type did not influence the products formed by this yeast, the composition of kombuchas 1 and 2 being similar. In treatment 3 (KMF at 1.10<sup>5</sup> UFC.mL<sup>-1</sup>), residual sugar concentration was higher, but it allowed LP to survive and produce 5 times more lactic acid (0.63 g.L<sup>-1</sup>) compared to other treatments. Again, the acetic acid bacteria (AA), did not survive, suggesting that many variables such as pH, oxygen availability, etc, can excerpt an influence over this bacterium. In all experiments, ethanol concentration (7.6 to 15.9 g.L<sup>-1</sup>) was higher than the legal limits for non-alcoholic beverages. The main role of acetic acid bacteria in kombucha is to oxidize ethanol to acetic acid, and because AA did not survive, the amounts of ethanol remained high in all formulations. Results showed that symbiosis among microorganisms is essential to the final product and the inocula size is crucial for this balance. Further studies are needed to ensure the viability of AA, in order to increase acetic acid production and decrease ethanol concentration.

Keywords: Kombucha production; starter culture; fermented beverage; yeasts; acetic acid bacteria.

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