Comprehensive Metabolomic Analysis of Kombucha: time-dependent chemical changes and biological effects in black tea, banana inflorescence, and grape juice driven by fermentation

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The Kombucha market has been growing in the last decade, the drink is commonly obtained by fermenting sweetened *Camellia sinensis* teas with a symbiotic culture of bacteria and yeast (SCOBY). The chemical profile can change through enzymatic degradation of the substrate and production of new molecules. Nevertheless, there's a lack of studies evaluating chemical responses of SCOBY when exposed to plants. Untargeted metabolomics can combine a robust set up for separation, detection and high-throughput data analysis assisted by software, which can be optimized and applied for chemical profiling of complex biological samples. Therefore, the present work applied a software assisted metabolomic workflow to investigate substrate-time related changes in chemical profile of 7-, 14- and 21 day-fermentation of kombucha derived from three extracts obtained from banana inflorescence (A/B), black tea (BT), and grape juice (GJ). The separation and data acquisition in positive and negative modes were performed using UPLC-ESI-MS, while MS-DIAL and MS-FINDER were used for data transformation and annotation. The web-based platform GNPS was used for determining metabolite spectral similarities through molecular networking. The biological implications of each extract were evaluated in DPPH-method for antioxidant activity and alpha-glucosidase inhibition for antihyperglycemic activity. The workflow enabled the annotation of 52 metabolites in BT extracts, 90 metabolites in A/B and 62 metabolites in GJ extracts. The microbial chemical responses were enzymatic hydrolysis, oxidation, and biosynthesis. In fermented black tea, gallic and dihydrosinapic acids were found as hydrolysis products alongside a sugar-derived product namely 7-(alpha-D-glucopyranosyloxy)-2,3,4,5,6-pentahydroxyheptanoic acid. The sphingolipids, safingol and cedefingol alongside capryloyl glycine and palmitoyl proline were identified. In fermented grape juice, sugar degradation and chemical transformation products were detected together with three cell membrane hopanoids. The fermented banana blossom showed the presence of one hopanoid structure, together with sphingofungin B, sphinganine and other fatty acid derivatives. The extracts presented different potencies in antioxidant and antihyperglycemic assays, providing insights of the substrate and time-related impact in kombucha fermentation. The present study is the first to apply a software-assisted metabolomic workflow to correlate different time-related kombucha fermented substrates together with chemical profiles and their biological implications, exploring and discussing the potential of kombucha as a functional food.

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