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Organic acids profile of water kefir beverages produced from pineapple waste extract

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The rinds, crowns and central axis of the pineapple are considered rejects by the fruit pulp industry. However, these residues contain nutrients, including hydrolysable sugars that can be used to produce fermented products such as water kefir beverages. Water kefir grains are made up of a consortium of lactic acid bacteria (LAB), acetic acid bacteria (AAB) and yeast that live in a stable symbiotic relationship. The main metabolites produced during the fermentation of water kefir are organic acids and ethanol. Organic acids are related to the protective effect of kefir beverages, in addition to being important for the sensory quality of the product.¹ Therefore, the objective of this research was to develop and evaluate the organic acid profile of two beverages fermented by water kefir grains, using pineapple residue extract, with and without guarana extract for 21 days at 4 °C.

To prepare the pineapple extract, the pineapple waste and water were mixed with a proportion of 2:1 (m/m), then 1.3% (m/m) of fresh mint was added to the mixture. All ingredients were mixed in a blender for 2 min. The final mixture was filtered and transferred to glass bottles. The bottles were pasteurized at 60 °C for 60 min and then cooled to 25 °C. To elaborate the formulation with guarana extract, 0.15% (m/m) guarana extract was added to the pineapple waste extract. To prepare the beverages, brown sugar was dissolved in water (10%, w/v). The sugar solution was sterilized and cooled to 25 °C. Three formulations were prepared: control (containing only the sugar solution) (FC), with the addition of pineapple waste extract (F1) and with the addition of pineapple waste extract containing guarana extract (F2). The fermentation process was carried out in two stages as described by Bueno et al.¹ Then the beverages were stored at 4 °C ± 1 °C in a refrigerator. Analyses were performed every 7 days for 21 days. For the analysis of organic acids, samples were diluted in a proportion of 1:3, followed by vortexing for one minute. Subsequently, the diluted sample was centrifuged at 15,000 rpm for 15 minutes. The supernatants were passed through Sep-Pak C18® and filtered through a 0.22 µm PVDF filter membrane. Organic acids were determined by High Performance Liquid Chromatography (HPLC) as described by Bueno et al.¹ The standards used were citric, malic, lactic, succinic, and acetic acids.

The variations found are attributed mainly to the addition of pineapple wastes (with and without guarana extracts) in the second fermentation step, as well as by the action of the diversified microbiota of the kefir grains capable of producing such acids.¹ In the beginning of second fermentation stage, citric acid was the major contributor in F1 and F2 beverages, probably derived from pineapple waste extracts. During storage, the profiles of all organic acid of the beverages reflected the metabolic heterogeneity of LAB and AAB. F1 formulation showed a progressive increase with the highest concentrations of acetic (2.46 ± 0.05 mg/mL), citric (4.83 ± 0.04 mg/mL), lactic (1.57 ± 0.05 mg/mL), and succinic (1.53 ± 0.03 mg/mL) acids verified on day 14, and subsequent decrease ($p < 0.05$) in the last day of cold storage. Similarly, Fiorda et al.² produced honey-water kefir and obtained citric acid as the main (69.86 mg/mL) organic acid, while lactic, acetic, and succinic were detected in smaller proportions.

In contrast, for F2 formulation, the acids contents increased more slowly, reaching a maximum (acetic, 3.03 ± 0.05 mg/mL; citric, 2.89 ± 0.01 mg/mL; succinic, 0.83 ± 0.00 mg/mL) at the end of the evaluated period. Only for lactic acid, this trend was not observed. The oxidative metabolism of AAB is evidenced mainly by the high concentrations of acetic acid in F2 beverage (21 day). The decrease of all organic acid, in the final period of storage, is observed mainly in F1 and can be explained by overoxidation of these acids to CO₂ and H₂O by the most acid tolerant genera, *Acetobacter* and *Komagataeibacter*. The difference in organic acid concentrations between beverages can also be attributed to the addition of guarana extract in F2. A recent study reported that the bioactive compounds present in these extracts demonstrate antimicrobial properties.³ Thus, the guarana extracts may be limiting the growth of microorganisms of F2 beverage, in addition to reducing the rates of organic acids production.

In conclusion, the use of alternative substrates, such as pineapple waste and guarana extract, for the development of kefir-fermented beverages, demonstrated to be an effective way to convert sugars into organic acids. Alternative substrates can help maintain and/or improve the food safety, nutrition, sensory and shelf-life properties of fermented beverages.

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