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Development of an Optimised Functional Food Enriched with Phenolic and Flavonoid Compounds from *Moringa Oleifera* leaves, Kefir Milk and Tualang honey Using Response Surface Methodology

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Structured Abstract

Background: *Moringa oleifera*, kefir milk, and Tualang honey are widely used as key ingredients for functional foods. Despite their high levels of polyphenols and flavonoids, the synergistic biological effects of their combination have not been extensively studied. The study aimed to develop optimised functional beverage formulations combining *M. oleifera*, kefir milk, and Tualang honey (MKT) with enhanced total phenolic and flavonoid content. The response surface methodology (RSM) based on a 3 factorial (3^3) Box-Behnken design (BBD) was used to determine the experimental design and the optimal ratio of the three ingredients.

Methods: The total phenolic and flavonoid contents of the designed formulations were assessed using the Folin-Ciocalteu and aluminium chloride colourimetric assays, respectively. MKT was analysed using the Design of Experiment (DOE), employing an RSM-based BBD to determine the optimal conditions for maximizing phenolic and flavonoid content.

Results: Formulation 8 exhibited the highest values of TPC (0.404 mg/ml) and TFC (0.117 mg/ml). A quadratic model indicated a strong correlation between ingredient ratios and bioactive content, with R^2 values of 0.7650 for TPC and 0.7314 for TFC.

Conclusion: In conclusion, Formulation 8 has been identified to be optimal formulation for maximizing total phenolic and flavonoid content, effectively enhancing the health benefits of the functional foods.

Keywords: Kefir milk, *Moringa oleifera*, tualang honey, response surface methodology, box-behken design

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