UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF AN ARTISANAL FERMENTATION: CASE STUDIES ON *CARICA PAPAYA* LEAF AND *GARCINIA MANGOSTANA* PERICARP

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PhD

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Fermentation is a green technique to enhance the bioavailability of many phenolic-rich medicinal plants. In this study, an artisanal fermentation technique was developed and applied on *Carica papaya* leaf (CPL) and *Garcinia mangostana* pericarp (GMP) to enhance the bioavailability of the materials. The study aimed to evaluate the effects of the fermentation technique on the total phenolic content (TPC) and antioxidant capacity of the plant models, identify the potential bioactive compounds of the fermented products, elucidate the population dynamics and identities of the microorganisms, characterise the toxicity of the fermented products and simulate the economic viability of an industrial scale fermentation process. TPC and antioxidant capacity of plant extracts were spectrophotometrically measured by Follin & Ciocalteu's and 2,2-diphenyl-2-picryl-hydrazyl (DPPH) reagents, respectively, followed by bioactive compounds identification by UHPLC-ESI-TOF-MS analysis. Population dynamics and identities of the microorganism were analysed by culture-dependent and metagenomic methods, respectively where the latter was based on the sequence homology of ribosomal genomes i.e. 16S rDNA, 5.8S-ITS rDNA and 26S rDNA of the microorganisms. The toxicity of the fermented materials was characterized by their lethal effects on zebrafish embryo at various concentrations. The economic viability of industrial-scale fermentation process was simulated by SuperPro Designer® software. The beneficial effects of the fermentation were observed in terms of enhanced TPC of the fermented CPL (of 5-L fermenter origin) at 48.42±0.31 mg GAE/g dry mass vs 12.13±0.39 mg GAE/g dry mass of the unfermented extract, whereas the antioxidant capacity of the fermented CPL was 467.38±4.09 mM TE/g dry mass, higher than the unfermented CPL i.e. 275.46±3.09 mM TE/g dry mass. Likewise, the enhancement of these two aspects were also observed on 50-L setup. Analysis on microbial population dynamics highlighted the prevalence of presumptive lactic acid bacteria (LAB) which later identified as *Lactobacillus* sepecies by DNA fingerprinting, and inhibition of presumptive enterobacteria in both 5-L and 50-L setups. Replicating the fermentation technique on GMP displayed strong reminiscence of its CPL counterpart in terms of enhanced total and antioxidant capacity of fermented GMP, prevalence of presumptive LAB and inhibition of presumptive enterobacteria. *Lactobacillus plantarum* and *Enterococcus faecalis* were amongst frequently LAB species detected in 5-L and 50-L ecosystems, respectively. Fermented CPL displayed higher acute toxicity effect (LC_{50}) than fermented GMP i.e. 133.1 µg/mL vs 100.2 µg/mL, respectively. Selected feedforward ANN models with embedded Levenberg-Marquardt algorithm and hyperbolic tangent sigmoid transfer function demonstrated statistical robustness in predicting the process responses. Process simulation at industrial scale on debottlenecked staggered configuration, demonstrated the economic viability of the fermentation process as indicated by the highest ROI (49.96%) and shorter payback period (2.26 years). In conclusion, this study revealed the benefit of the artisanal fermentation in enhancing the functionalities of the plant materials, while yielding potential bioactive compounds, cultivating potential probiotic species and inhibiting potential pathogens. The consistensies of these indicators across different plant models and production scales proved the reproducibility and scalability of the fermentation technique. The industrial scale fermentation process was also economically viable if the fermentation stage was implemented in staggered configuration.

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