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POTENTIAL OF FRUIT WASTE IN PROMOTING IN VITRO PROBIOTIC GROWTH

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POTENTIAL OF FRUIT WASTE IN PROMOTING *IN VITRO* PROBIOTIC GROWTH

By

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POTENTIAL OF FRUIT WASTE IN PROMOTING *IN VITRO* PROBIOTIC GROWTH

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Chair : Leong Sui Sien, PhD
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Fruit waste (FW) refers to the loss and waste of unneeded or unconsumed components of fruit that are biodegradable. This includes the fruit peel, pulp, seeds, and leaves. FW was reported as a non-conventional alternative source of nutritional and mineral content that might be employed as functional food components. However, the prebiotic significance of FW is poorly recognized. This limitation is overcome by the invention of FW as a prebiotic source through *in vitro* fermentation on *Lactobacillus casei Shirota* (*L. casei S*.) growth. Therefore, the objectives of this study were to investigate the nutritional and mineral composition of FW from banana, orange, and watermelon peels, determine the growth and survival of probiotics using FW as a substrate, and assess the prebiotic potential and metabolites produced from FW through *in vitro* monoculture fermentation. In this study, the peel cuts were boiled in hot water (90°C) for 30 minutes to inactivate any potential microorganisms and enzyme reactions. The peels were then processed separately into two groups: freeze-dried (FD) and oven-dried (OD). The nutritional properties, mineral composition, and prebiotic activities of FW were analyzed. Both drying techniques were shown to be quite effective in preserving and reducing the FW moisture. Furthermore, the carbohydrate content ranged from 47.74–83.04% dry weight basis, with crude fibre content supplied in greater quantities. There was a wide variety of mineral content detected, with calcium and sodium being the most abundantly generated compounds. It was found that *L. casei S.* growth in modified de Man Rogosa Sharpe (mMRS) broth enrinched with FW from banana indicated a higher bacterial count and survival rate in comparison with inulin, which ranged from 91.61–98.66%. Thus, it presents an excellent potential substrate for probiotics. Further on, as a prebiotic ingredient, the growth density of *L. casei S.* through *in vitro* monoculture fermentation showed a positive prebiotic effect in FW which is higher than inulin, which acts as a positive control. Samples obtained at 0, 6, 12, and 24 hours were evaluated for organic acid production by high-performance liquid chromatography (HPLC), resulting in positive short

chain fatty acid (SCFA) production, with butyrate being the major organic acid generated. The study discovered that FW has the potential to enhance probiotic development, act as a source of fermentable substrate, and produce beneficial organic compounds. Thus, it will be able to function as an alternative prebiotic ingredient, which could be a significant contributor to novel functional food applications on a commercial scale.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

POTENSI SISA BUAH DALAM MERANSANG PERTUMBUHAN PROBIOTIK SECARA *IN VITRO*

Oleh

ERRA FAZIRA BINTI ABDUL RAHIM

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Sisa buah (FW) merujuk kepada kehilangan dan pembaziran komponen buah yang tidak diperlukan atau tidak digunakan yang boleh dikitar semula. Ini merangkumi kulit buah, pulpa, biji, dan daun. FW telah dilaporkan sebagai sumber alternatif bukan konvensional bagi sumber kandungan nutrisi dan mineral yang dapat digunakan sebagai komponen makanan berfungsi. Walaubagaimanapun, kepentingan prebiotik dalam FW ini kurang diakui. Masalah ini diatasi dengan menjadikan FW sebagai sumber prebiotik melalui proses penapaian *in vitro* ke atas pertumbuhan *Lactobacillus casei Shirota* (*L. casei S*.). Oleh itu, objektif kajian ini adalah untuk mengkaji komposisi nutrisi dan mineral dalam FW daripada buah pisang, oren dan tembikai, menentukan pertumbuhan dan kadar ketahanan hidup probiotik dengan menggunakan FW sebagai sumber substrat utama, dan juga menilai potensi prebiotik dan penghasilan metabolit menggunakan proses penapaian *in vitro* secara monokultur. Dalam kajian ini, FW telah dibersihkan dengan mendidihkan kulit buah yang telah dipotong ke dalam air panas (± 90°C) selama 30 minit bagi menyahaktifkan sebarang potensi tindak balas mikroorganisma dan reaksi enzim. Seterusnya, kulit buah tersebut diproses secara berasingan, iaitu dikeringkan di dalam pengering beku (FD) dan ketuhar (OD). Selepas itu, analisis sifat nutrisi, komposisi mineral, dan aktiviti prebiotik dalam FW telah dijalankan. Kedua-dua teknik pengeringan telah terbukti berkesan dalam penyimpanan dan mengurangkan kadar kelembapan dalam FW. Selain itu, terdapat perbezaan kandungan karbohidrat bagi setiap FW iaitu bermula dari 47.74 hingga 83.04% kadar berat kering, di mana sebahagian besar kuantiti nutrisi dihasilkan oleh serat mentah. Terdapat juga pelbagai jenis kandungan mineral yang telah dikenal pasti, di mana sebahagian besarnya terdiri daripada kalsium dan natrium. Didapati bahawa pertumbuhan *L. casei S*. dalam media De Man, Rogosa dan Sharpe (mMRS) yang ditambah dengan sampel pisang telah menunjukkan bilangan bakteria dan kadar kelangsungan hidup yang lebih tinggi berbanding inulin yang berperanan sebagai kawalan positif, iaitu antara 91.61 hingga 98.66%. Oleh itu, ia telah menampilkan sebagai potensi substrat yang

sangat baik untuk probiotik. Selanjutnya sebagai bahan prebiotik, ketumpatan pertumbuhan *L. casei S.* melalui penapaian *in vitro* secara monokultur telah menunjukkan kesan positif dalam FW yang mana lebih tinggi daripada inulin yang bertindak sebagai kawalan positif. Sampel penapaian yang diperoleh pada 0, 6, 12, dan 24 jam telah dinilai bagi analisis asid organik dengan menggunakan *high-performance liquid chromatography* (HPLC) dan turut menunjukkan kadar produksi *short chain fatty acid* (SCFA) yang positif dengan butirat sebagai asid organik utama yang dihasilkan. Kajian mendapati bahawa FW berpotensi untuk meningkatkan pertumbuhan probiotik, sumber substrat yang boleh ditapai, dan menghasilkan sebatian organik yang berfaedah, oleh itu FW mampu berfungsi sebagai bahan prebiotik alternatif yang boleh menyumbang kepada aplikasi makanan fungsi yang inovatif pada skala komersial.

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I certify that a Thesis Examination Committee has met on 28 October 2021 to conduct the final examination of Erra Fazira binti Abdul Rahim on her thesis entitled "Potential of Fruit Waste in Promoting *In Vitro* Probiotic Growth" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
FAO	World and Agriculture Organization
FD	Freeze-dried
FW	Fruit waste
GRAS	Generally Recognized as Safe
HPLC	High-performance liquid chromatography
OD	Oven-dried
OD650	Optical density
RDA	Recommended Dietary Allowances
SCFA	Short chain fatty acid

CHAPTER 1

INTRODUCTION

1.1 Study Background

Fruit waste (FW) is the loss and waste of biodegradable components of fruit that are unnecessary or unconsumed. This contains the fruit's peel, skin, pulp, seeds, and leaves (Oorxax, 2009). FW disposal is classified as solid waste disposal, which, according to the Malaysia Solid Waste and Public Cleansing Management Act 2007 (Act 672), may include any approach involving combustion, incineration, deposition, or decomposition (Ngapan et al., 2012). In Thailand, 15% of FW was digested into bio-products such as biogas and bio-fertilizer (Pollution Control Department, 2010). However, composting food waste is less successful in some developing nations for a variety of reasons, including incorrect separation of food waste from other solid waste and a lack of a management system (Thi et al., 2015). Landfill and incineration are the most common ways to get rid of FW. Unfortunately, waste disposal through landfills has grown increasingly challenging in Malaysia since many landfills have surpassed their capacity (Moh & Manaf, 2014). Meanwhile, incineration is seldom used to handle FW since it contributes to air pollution (Zhang et al., 2014). Because of this, there is a need for a cost-effective and environmentally friendly way to handle and manage FW. Composting, anaerobic digestion, and animal feeding are the most prevalent and environmentally favourable techniques of FW management. These are sustainable food waste management strategies suited for future adoption in Malaysia (Thi et al., 2015).

It is difficult to locate any research articles on FW figures in Malaysia since the majority of newspapers classify FW as municipal solid waste (MSW) (Siti Wahidah, 2017). Shifting food waste from landfills would not only save landfill space but also help reduce greenhouse gas emissions. Anaerobic digestion has been identified as a cost-effective and ecologically friendly alternative to FW. This technology provides a number of qualitative advantages, such as lowering the volume of MSW, lowering the transportation cost of delivering MSW to the landfill, lowering landfill emissions and leachate, extending the life of the landfill, and minimising land usage (MEAKO, 2015). Malaysia's sewerage industry may be technologically enhanced by introducing an anaerobic digestion system into mechanised treatment plants, which is a prospective source of biogas produced from sewage treatment plant waste (Kumaran et al., 2016).

Today, several scientific studies on FW provide evidence to support the claims of FW as a great source of dietary fibre and prebiotics in the preparation of functional food. For instance, pectins extracted from apple (*Malusdomestica*), lemon (*Citrus limon*), and orange (*Citrus sinensis*) peels enhanced the probiotic growth of *Bifidobacterium bifidum* and *Lactobacillus* (*L.*) *acidophilus* in the human gastrointestinal tract at extremely acidic to alkaline conditions by Sen et

al., (2014). Whereas, pineapple (*Ananas comosus* [L.] Merrill) peel and pomace were also shown to have prebiotic effects on *L. acidophilus, L. casei, and L. paracasei spp. paracasei* due to their nutritional properties of dietary fibres, sugars, proteins, and minerals (Sah et al., 2016). Therefore, the potential of FW is not only limited to the production of value-added products but can also be used in promoting probiotic bacteria and serves as an efficient practise of waste management (Sen et al., 2014). However, as a prebiotic potential ingredient, less study was reported, notably regarding the *Lactobacillus* growth effect and metabolite production. *Lactobacillus* was chosen for this study because it is often used in manufacturing and costs less than other lactic acid bacteria.

Previously, a few studies have shown that FW promotes probiotic development but is rarely addressed for SCFA synthesis. Therefore, this study was conducted based on the assumption that the potential prebiotic found in FW could be a valuable contribution in new functional food formulations to enhance probiotic activities and organic acid production through *in vitro* monoculture fermentation due to its nutritional and mineral bioavailability.

1.2 Objectives

The objectives of this study were:

a. To investigate the nutritional and mineral composition of the FW, particularly in banana (*Musa acuminata*), orange (*Citrus reticulata*), and watermelon (*Citrullus lanatus*).
b. To determine the growth and survival of *L. casei S.* on FW as a substrate.
c. To assess the effect of prebiotic potential and organic acid production from FW through *in vitro* fermentation on *L. casei S*. growth.

1.3 Hypotheses

The hypotheses of this study include:

a. Fruit waste causes economic and environmental impacts and also indirectly includes the wasting of critical resources due to improper disposal processes. The FW as a valuable nutritional and functional food ingredient may be a potential solution, but it has yet to be studied.
b. Probiotic strain such as *Lactobacillus* are expected to reveal positive growth toward FW as a potential substrate.
c. *In vitro* fermentation is believed to produce positive *Lactobacillus* growth and significant production of short chain fatty acids by using FW as a potential prebiotic ingredient.

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