BIOTRANSFORMATION OF GLUTEN-FREE COMPOSITE FLOUR MEDIATED BY LACTIC ACID BACTERIA VIA SOLID-STATE FERMENTATION PROCESS CONDUCTED UNDER DIFFERENT MOISTURE CONTENTS

By

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Over the years, the development of staple food products from composite flour is considered a low-cost approach to address the burden of protein-energy malnutrition in developing countries. However, despite the numerous advantages of composite flour, the high amount of anti-nutritional factors typical to food derived from plant origin usually impede nutrient bioavailability. Five strains of lactic acid bacteria (LAB) isolated from Malaysian foods that include *Lactiplantibacillus plantarum* RG-14, *L. plantarum* RI-11, *L. plantarum* RS5, *L. plantarum* IUL-4, and *Pediococcus pentosaceus* UP-2 have been reported for their capabilities to produce various extracellular hydrolytic enzymes via solid-state fermentation (SSF) which can breakdown complex food matrix into smaller absorbable forms and reduced antinutrients. Therefore, the LAB strains were employed in this study to biotransform the gluten-free composite flour derived from rice, sorghum, and soybean. The SSF process was performed under 30-60% moisture content for 7 days, where samples were withdrawn at 24 h intervals for various analyses such as LAB cell viability, pH, total titratable acidity, extracellular protease activity, soluble protein concentration, crude protein content, and *in vitro* protein digestibility. The pH of the biotransformed composite flour showed a significant reduction from the initial range of pH 5.98 - 6.67 to the final pH of 4.36 - 3.65, corresponding to the increase in the percentage of total titratable acidity in the range of 0.28 - 0.47% to 1.07 - 1.65% from Day 0-4 and remained stable till Day 7 of the SSF process. The LAB strains exhibited high extracellular proteolytic activity (0.63 - 1.35 U/mg to 4.21 - 5.13 U/mg) from Day 0-7. In addition, the treated composite flour soluble protein increased significantly ($p \leq 0.05$) (0.58 - 0.60 mg/mL to 0.72 - 0.79 mg/mL) from Day 0-7, crude protein content (12.00 - 12.18% to 13.04 - 14.39%) and protein digestibility (70.05 - 70.72% to 78.46 - 79.95%) from Day 0-4 of SSF. In addition, the antinutritional factors of the biotransformed composite flour showed a significant reduction ($p \leq 0.05$) in the phytic acid (127.11 – 137.73 mg/100 g to 124.84 -

120.24 mg/100 g) and tannin content (89.48 – 93.92 mg/100 g to 63.51 – 39.84 mg/100g). Since lower moisture content promotes flour quality, 50% moisture was selected as the most suitable moisture content to have effectively biotransformed the composite flour, even though a comparable result was observed at 60% moisture content. Overall, *Lactiplantibacillus plantarum* RG-14 was ranked the best strain attributed to the general improvement in composite flour's pH, TTA, protein quality, and antinutritional properties.

Keywords: composite flour; lactic acid bacteria; solid-state fermentation; moisture content; nutritive quality

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

BIOTRANSFORMASI TEPUNG KOMPOSIT BEBAS GLUTEN DIMEDIASI BAKTERIA ASID LAKTIK PENAPAIAN PEPEJAL YANG DIJALANKAN DI BAWAH KANDUNGAN KELEMBAPAN YANG BERBEZA

Oleh

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Pembangunan produk makanan ruji daripada tepung komposit dari tahun ke tahun dianggap sebagai pendekatan kos rendah untuk menangani beban malnutrisi tenaga protein di negara membangun. Walaupun terdapat banyak kelebihan tepung komposit, namun jumlah faktor anti-pemakanan yang tinggi dan tipikal kepada makanan yang diperoleh daripada asal tumbuhan biasanya menghalang bioavailabiliti nutrien. Lima strain bakteria asid laktik (LAB) iaitu *Lactiplantibacillus plantarum* RG-14, *L. plantarum* RI-11, *L. plantarum* RS5, *L. plantarum* IUL-4, dan *Pediococcus pentosaceus* UP-2 yang telah diasingkan daripada makanan negara Malaysia dilaporkan mempunyai keupayaan untuk menghasilkan pelbagai enzim hidrolitik ekstraselular melalui penapaian keadaan pepejal (SSF) yang boleh memecahkan matrik makanan kompleks kepada bentuk yang lebih kecil dan mudah diserap serta mengurangkan antinutrien. Oleh itu, strain LAB telah digunakan dalam kajian ini untuk biotransformasi tepung komposit bebas gluten yang diperoleh daripada beras, sekoi dan kacang soya. Kaedah SSF dilakukan di bawah 30-60% kandungan lembapan dan dijalankan selama 7 hari. Sampel diambil pada selang 24 jam untuk analisis pH, jumlah keasidan boleh titrasi, aktiviti protease ekstraselular, kepekatan protein larut, kandungan protein kasar, dan kebolehcernaan protein *in vitro*. Nilai pH tepung komposit biotransformasi menunjukkan pengurangan yang ketara, dari nilai pH permulaan 5.98 – 6.67 ke nilai pH akhiran 4.36 – 3.65, sepadan dengan peningkatan peratusan jumlah keasidan boleh titrasi (0.28 – 0.47% ke 1.07 – 1.65%) yang dicatatkan pada hari pertama hingga hari keempat, dan nilai peratusan kekal stabil sehingga hari ketujuh. Strain LAB menunjukkan aktiviti proteolitik ekstraselular yang tinggi (0.63 - 1.35 U/mg ke 4.21 - 5.13 U/mg) dari hari pertama hingga hari ketujuh. Selain itu, nilai peningkatan yang ketara ditunjukkan pada hari pertama hingga hari ketujuh pada protein larut tepung komposit yang dirawat ($p \leq 0.05$) (0.58 - 0.60 mg/mL kepada 0.72 - 0.79 mg/mL), dan kandungan protein kasar (12.00 - 12.18% kepada 13.04 - 14.39%) serta kebolehcernaan protein (70.05 - 70.72% kepada 78.46 - 79.95%) pada hari pertama ke hari keempat ujian SSF dijalankan. Faktor antinutrisi tepung komposit biotransformasi menunjukkan pengurangan ketara ($p \leq 0.05$) dalam asid fitak (127.11 – 137.73 mg/100 g kepada 124.84 - 120.24 mg/100 g) dan kandungan tanin (89.48 – 93. 100 g hingga 63.51 – 39.84 mg/100g). Kandungan lembapan yang lebih rendah menggalakkan kualiti tepung,dan sebanyak 50% lembapan telah dipilih sebagai kandungan lembapan yang paling sesuai untuk mentransformasikan tepung komposit secara berkesan, walaupun hasil yang setanding diperhatikan pada kandungan lembapan 60%. Secara keseluruhan, Lactiplantibacillus plantarum RG-14 disenaraikan sebagai strain terbaik yang dikaitkan dengan peningkatan umum dalam pH tepung komposit, TTA, kualiti protein dan sifat antinutrisi.

Kata Kunci: tepung komposit; bakteria asid laktik; penapaian keadaan pepejal; kandungan lembapan; kualiti pemakanan

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BSA	bovine serum albumin
CFU	colony forming unit
EMB	eosin methylene blue agar
HCl	hydrochloric acid
IUL-4	*Lactiplantibacillus plantarum* IUL-4
IVPD	*in vitro* protein digestibility
LAB	lactic acid bacteria
MC	moisture content
MRS	de man, rogosa sharpe medium agar
NTC	raw composite flour with no water and culture
PEM	protein-energy malnutrition
PKC	palm kernel cake
RG-14	*Lactiplantibacillus plantarum* RG-14
RI-11	*Lactiplantibacillus plantarum* RI-11
RS-5	*Lactiplantibacillus plantarum* RS-5
RTU	ready to use culture
SDB	sabouraud dextrose agar
SMF	submerged fermentation
SSF	solid-state fermentation
TCA	trichloroacetic acid
TTA	total titratable acidity
UP-2	*Pediococcus pentosaceus* UP-2
WTC	water-treated composite with no culture

CHAPTER 1

INTRODUCTION

Malnutrition resulting from protein-energy deficiencies remains a major health burden in developing countries with approximately 821 million people including 667 million children currently undernourished (FAO/WHO, 2019). Protein-energy malnutrition (PEM) occurs due to insufficient intake of quality protein and the symptoms include marasmus, kwashiorkor, stunted growth, and underweight. According to the global prevalence report on PEM, Africa and Asia had the highest occurrence of the health burden (FAO, 2018). Similarly, several studies have established the consumption of low-quality diets as one of the major factors responsible for the high PEM in developing countries (Adesogan et al., 2019; Nazri et al., 2020). Food grains obtained from cereals and legumes provide the cheapest source of dietary energy such as protein, fat, vitamins, and minerals for people in developing countries (Gilani et al., 2012; Udomkun et al., 2019). According to the global statistics report for cereals and legumes consumption, nearly 3.5 billion people consume rice (FAO, 2016), about 300 million people in Africa depend on sorghum-based staple foods while millions of people globally relied on soybean (Dicko et al, 2006; FAO, 2019). Although rice, sorghum, and soybean are cheap and accessible to vulnerable populations, over-reliance on a single type of food (monotonous diet) can lead to nutritional deficiencies (Govindaraj, 2015), thus, suggesting composting cereals and legumes to produce nutritious foods (Temba et al., 2016).

In 1964, the Food and Agricultural Organization (FAO) introduce the concept of composite flour technology as an attempt to reduce the financial burden of wheat importation in developing countries (Hasmadi et al., 2020). Composite flour involves the mixture of wheat with varying proportions of legumes, tubers, cereals, and pseudocereals to develop food products (Melini et al., 2020). However, the major concern for wheat usage in food production is the presence of gluten protein which has been linked to the incidence of celiac disease with a global prevalence rate of 2.4% to 44% (Bolarinwa & Oyesiji, 2021; Mahadev & Green, 2011). Hence, recent research focuses on the development of non-wheat composite flour for use in food production (Melini et al., 2017). Rice, sorghum, and soybean are gluten-free and widely consumed in developing countries. Thus, the development of protein-rich composite flour from rice, sorghum, and soybean grains offers a vast potential to alleviate PEM. Food products made from composite flour derived from rice, sorghum, and soybean have been reported to possess features capable of replacing the wheat-based composite (Adeyeye, 2018; Adeyeye et al., 2017; Seth & Rajamanickam, 2012). However, despite the low-cost and high accessibility advantage of composite flour derived from rice, sorghum, and soybean, staple foods derived from plant sources contains a high amount of antinutritional factors that impede nutrient bioavailability (Schönfeldt & Hall, 2012; Udomkun et al., 2019).

Protein quality interrelates with protein digestibility (Boye et al., 2012) and research efforts over the years on composite flour have focused on improving the protein content/lysine limitations in cereal-based food products. A study on food products developed from rice, sorghum, millet, and soybean composite flour (Seth & Rajamanickam, 2012), rice, sorghum, and soybean composite flour (Omwamba & Mahungu, 2014) via extrusion processing method recorded a significant loss in lysine content and does not account for protein digestibility and anti-nutritional factors in composite flour. Food processing is meant to improve nutritive quality in food products (Joye, 2019) and the biotransformation process mediated by wholly microorganisms via solid-state fermentation technique (SSF) holds a promising potential to improve food quality.

SSF technique is a bioprocess carried out in the absence or near-absence of free water, although, the moisture content of the substrate must be sufficient to support microbial growth and metabolic activity (Thomas et al., 2013). SSF is eco-friendly, energy-efficient, low risk of contamination risk, and high bioproducts productivity (Thomas et al., 2013; Webb & Manan, 2017). Apart from moisture content being an important criterion to develop the SSF process, the choice of microorganisms also plays a significant role in determining the success of the bioprocess (Thomas et al., 2013). Fungi are considered the ideal microorganisms for SSF since they mimic their natural habitat, however, the possibilities of mycotoxin contamination have limited fungal application in food production (Adebiyi et al., 2019). Contrarily, bacteria such as the lactic acid bacteria (LAB) that are known to exhibit limited growth under SSF conditions are currently being applied as biotransformation agents for many bioprocesses to develop bioproducts from cheap agricultural biomass (Lee et al., 2019).

LAB are Gram-positive rod or cocci non-respiring, aerotolerant, fastidious, catalase-negative, acid-tolerant, and nonsporulating bacteria. They are generally referred as safe (GRAS) and are widely known for their functional role in food fermentation (Zabidi et al., 2020). In this study, a few strains of LAB such as *Lactiplantibacillus plantarum* RI-11, *L. plantarum* RG-14, *L. plantarum* RS-5, *L. plantarum* IUL-4, and *Pediococcus pentocaseous* UP-2 isolated from Malaysian foods were recently reported for their capabilities to induce extracellular hydrolytic enzymes under SSF conditions (Lee et al., 2019). The extracellular hydrolytic enzymes induced by the LAB are capable of breaking down complex food matrix into digestible forms (Kårlund et al., 2020). However, previous application of these LAB and their postbiotic metabolites have focused on improving feed quality (Lee et al., 2019) and livestock health (Izuddin et al., 2020; Merzza et al., 2019). Therefore, the current study aimed at evaluating the effects of moisture contents on the SSF of composite flour derived from rice sorghum and soybean mediated by the selected LAB via the following specific objectives:

i. To determine the cell viability of selected LAB, pH, and total titratable acidity of biotransformed gluten-free composite flour mediated by selected LAB under different moisture contents.

ii. To determine the extracellular protease activity, protein concentration and protein digestibility of biotransformed gluten-free composite flour mediated by selected LAB under different moisture contents.

iii. To determine the antinutritional factors of biotransformed gluten-free composite flour mediated by selected LAB under different moisture contents.

1.1 Research Hypothesis

Rice and sorghum basically contain low protein. Soybean is cheaper protein source compared to animal origin food. The protein content of gluten-free composite flour derived from rice and sorghum can be improved by adding soybean flour. However, the presence of antinutritional factors in the composite flour usually affect the protein digestibility. Thus, to further improve the protein content/digestibility of the gluten-free composite flour the following hypothesis were proposed:

Ho - Biotransformation process mediated via SSF by various lactic acid bacteria isolated from Malaysian foods will further enhance the protein content/digestibility of gluten-free composite flour

Ho - Moisture content will have significant effect on the biotransformation of gluten-free composite flour mediated via SSF by various lactic acid bacteria isolated from Malaysian foods

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