EFFECTS OF SHORT-TERM MICROBIAL FERMENTATION OF INDUSTRIAL POTATO WASTE ON CHEMICAL CONTENTS AND RUMINAL *In Vitro* FERMENTATION CHARACTERISTICS

By

MUHAMMAD SURAJO AFAKA

Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To my mother, Hajiya Salamatu Muhammad Sani.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chair : Associate Professor Halimatun binti Yaakub, PhD
Faculty : Agriculture

Potato processing generates waste in the form of peels, pulp and rejects which is estimated to be around 12-20% of their total production volume. Potato peels, pulp and unmarketable potatoes can further be processed in starch plants, incorporated into animal feed formulations, or turned into ethanol. However, there is limited nutritional information on the potential of industrial potato waste (IPW) as ruminant feed. Thus, this research aimed to validate and characterize experimental isolates, determine chemical composition and nutritive values of inoculated and fermented IPW, determine antinutritional factors (ANF), glycoalkaloid (GLA), and antioxidant capacity, and finally determine *in vitro* digestibility, microbial population, and rumen fermentation characteristics to up-cycle the industrial potato waste which will provide additional feed options for the livestock and make potato growing and processing more economical. The research design involved uninoculated IPW (control) and IPW inoculated with *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae*, and *Aspergillus oryzae*; thus each inoculated IPW served as a treatment. The treatments were subjected to fermentation at 0 (control), 24, 48, and 72 h. The layout of the experiment was a completely randomized (4 × 4) design (CRD) with factorial arrangement. In the first study, three isolates used for inoculation of IPW were characterized and confirmed as *L. plantarum*, *S. cerevisiae*, and *A. oryzae* with discrete accession numbers of MW296876, MW296931, and MW297015, respectively. The control, *L. plantarum and S. cerevisiae* treatments recorded gross energy (15.59 – 15.87 MJ/kg DM) higher ($p<0.05$) than 15.42 – 15.61 MJ/kg DM recorded for *A. oryzae* treatment. It was discovered that antioxidant capacity increased (33.94 – 41.39 Trolox Eq. mg/g) significantly ($p<0.05$) across the fermentation time (h) until 48 h; thereafter the antioxidant capacity dropped (27.02 – 35.94 Trolox Eq. mg/g). In the final study, treatment and fermentation time had no effect ($p>0.05$) on *in vitro* dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), and metabolizable energy (ME). Although the methane content (7.11±1.49 – 8.07±0.32 mM) of *A. oryzae* did not changed across the fermentation time, the values recorded therein were significantly $p<0.05$) the lowest compared to other treatments with a range of 7.77 – 13.03 mM.

Results on biohydrogenation revealed that IPW fermented with *A. oryzae* recorded significantly ($p<0.05$) highest concentration (1299.40 - 2085.29 µg/100 mL) of C18:0 (stearic acid) across all the fermentation time compared to 370.62 - 651.93 µg/100 mL recorded in the control, *L. plantarum* and *S. cerevisiae*. Consequent to the results of this study, it was concluded that inoculation of IPW improves nutritive values, phenolic compounds, antioxidant capacity, and biohydrogenation; as well as the reduction in solanine content and methane gas production. Among the microbes used in this study, *A. oryzae* is highly recommended because it recorded the highest content of stearic acid via the biohydrogenation process, and it reduced methane gas production than the control and IPW inoculated with *L. plantarum* and *S. cerevisiae*.

Keywords: Phytochemical compounds, Antioxidants, Glycoalkaloids, Biohydrogenation, Methane reduction.

SDG: GOAL 12: Responsible Consumption and Production

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PENAPAIAN MIKROB JANGKA PENDEK SISA KENTANG INDUSTRI TERHADAP KANDUNGAN KIMIA DAN CIRI-CIRI PENAPAIAN IN VITRO RUMINAL

Oleh

MUHAMMAD SURAJO AFAKA

Oktober 2023

Pengerusi : Profesor Madya Halimatun binti Yaakub, PhD
Fakulti : Pertanian

Kaedah memproses kentang menghasilkan bahan buangan di dalam bentuk kulit, pulpa dan bahan buangan ini dianggarkan merangkumi sebanyak 12-20% daripada jumlah isipadu produksi keseluruhan. Kulit, pulpa dan kentang yang tidak dapat dipasarkan boleh diproses selanjutnya di kilang pemprosesan kanji, seterusnya digabungkan ke dalam formulasi makanan haiwan, atau ditukarkan kepada etanol. Namun terdapat kekurangan informasi mengenai nutrisi berkenaan potensi bahan buangan industri kentang (IPW) sebagai makanan ruminan. Justeru, kajian ini telah dijalankan untuk mengesahkan dan mengklasifikasikan karakter yang telah diasingkan di dalam eksperimen, untuk menentukan komposisi kimia dan nilai nutrisi IPW yang telah diinokulasi dan difermentasi, untuk menentukan faktor-faktor anti-nutrisi (ANF), glycoalkaloid (GLA), dan kapasiti antioksidan, dan yang terakhir untuk menentukan tahap penghadaman secara *in vitro*, populasi mikrob, dan karakteristik fermentasi rumen agar dapat meningkatkan kebolehgunaan bahan buangan industri kentang, yang mana ianya dapat menyediakan lebih banyak pilihan makanan untuk ternakan dan lantas menjadikan penanaman kentang dan kaedah pemprosesannya lebih ekonimikal. Rekabentuk kajian telah melibatkan IPW yang tidak diinokulasi (kawalan) dan IPW yang telah diinokulasi bersama *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae*, and *Aspergillus oryzae*; di mana setiap satu IPW yang telah diinokulasi bertindak sebagai kumpulan rawatan. Kumpulan rawatan telah defermentasi pada 0 (kawalan), 24, 48, dan 72 jam. Susunan eksperimen adalah secara rekabentuk rawak sepenuhnya (4 × 4) (CRD), dengan susunan faktorial. Di dalam kajian pertama, tiga isolat yang telah digunakan untuk inokulasi IPW telah melalui proses pengecaman karakteristik dan disahkan sebagai *L. plantarum*, *S. cerevisiae*, dan *A. oryzae* yang mempunyai nombor akses yang berbeza iaitu MW296876, MW296931, dan MW297015. Kumpulan kawalan, *L. plantarum* dan *S. cerevisiae* merekodkan tenaga kasar (15.59 – 15.87 MJ/kg DM) lebih tinggi ($p<0.05$) daripada 15.42 – 15.61 MJ/kg DM yang direkodkan untuk kumpulan rawatn *A.* oryzae. Kapasiti antioksidan telah dilihat meningkat (33.94 – 41.39 Trolox Eq. mg/g) secara signifikan ($p<0.05$) bagi kesemua tempah fermentasi (h) sehingga 48 jam; di mana setelah itu kapasiti antioksidan telah menurun (27.02 – 35.94 Trolox Eq. mg/g). Di dalam kajian akhir, kumpulan rawatan dan tempoh fermentasi tidak memberikan kesan ($p>0.05$) pada pencernaan bahan kering in vitro (IVDMD), pencernaan bahan organic in vitro (IVOMD), dan tenaga yang boleh dimetabolismakan (ME). Walaupun kandungan metana (7.11±1.49 – 8.07±0.32 mM) *A. oryzae* tidak berubah untuk kesemua tempoh fermentasi, nilai yang direkodkan adalah lebih rendah ($p<0.05$) berbanding kumpulan-kumpulan rawatan yang lain dengan julat 7.77 – 13.03 mM). Keputusan bagi biohidrogenasi menunjukkan bahawa IPW yang telah difermentasi dengan *A. oryzae* merekodkan peningkatan signifikan ($p<0.05$) (1299.40 – 2085.29 µg/100 mL) kepada C18:0 (asid sterik) bagi kesemua tempoh fermentasi berbanding 370.62 – 651.93 µg/100 mL yang direkodkan di dalam kumpulan kawalan, *L. plantarum* and *S. cerevisiae*. Hasil daripada dapatan kajian ini, ianya boleh disimpulkan bahawa IPW yang telah diinokulasikan meningkatkan nilai-nilai nutrisi, sebatian fenolik, kapasiti antioksidan, dan biohidrogenasi; yang juga penurunan kandungan solanine dan pengeluaran gas metana. Di kalangan mikrob yang telah digunakan di dalam kajian ini, *A. oryzae* adalah yang paling direkomendasi untuk digunakan kerana ianya merekodkan kandungan asid sterik yang tertinggi melalui proses biohidrogenasi, dan ianya menurunkan penghasilan gas metana berbanding kumpulan kawalan dan IPW yang telah diinokulasi dengan *L. plantarum* dan *S. cerevisiae*.

Kata kunci: Sebatian fitokimia, Antioksidan, Glikoalkaloid, Biohidrogenasi, Pengurangan metana.

SDG: MATLAMAT 12: Tanggungjawab Penggunaan dan Pengeluaran

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Halimatun binti Yaakub, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Iswan Budy bin Hj. Suyub, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Frisco bin Nobily, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

____

ZALILAH MOHD SHARIF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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LIST OF ABBREVIATIONS

°C	degree Celsius
µL	micro litter
µm	micro meter
µM	micromole
ADF	acid detergent fibre
ADL	acid detergent lignin
ANF	antinutritional factor
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
ATP	adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
cfu	colony forming unit
CP	crude protein
CRD	completely randomized design
DM	dry matter
DNA	deoxyribonucleic acid
DW	dry weight
ECPD	effective crude protein digestibility
EDTA	ethylenediaminetetracetic acid
EE	ether extract
Eq	equivalent
g	gram
gDNA	genomic deoxyribonucleic acid
GIT	gastrointestinal tract

GLA	glycoalkaloids
GLM	general linear model
GOP	total gross output
GP24	net gas production at 24 h of incubation
H/C	Hemicellulose
HPLC	high-performance liquid chromatography
IPW	Industrial potato waste
ITS	internal transcribed spacer
IVDMD	in vitro dry matter digestibility
IVOMD	in vitro organic matter digestibility
Kg	kilogram
KK	Kedah-Kelantan
LA	lactic acid
LCD	liquid-crystal display
MARDI	Malaysian Agricultural Research and Development Institute
MBY	microbial biomass yield
MCL	Maximum Composite Likelihood
ME	metabolizable energy
MEGA	Molecular Evolutionary Genetics Analysis
mg	milligram
MJ	mega joule
mL	millilitre
mm	millimetre
mmol	millimole
MRS	de Man, Rogosa and Sharpe

MRVP	Methyl red and Voges Proskauer
MUFA	monounsaturated fatty acids
NBS	National Bureau of Standard
NCBI	National Centre for Biotechnology Information
NDF	Neutral detergent fibre
NDS	Neutral detergent solution
NIRS	near infra-red reflectance spectroscopy
OM	organic matter
p	probability
PCR	polymerase chain reaction
PDA	potato dextrose agar
PKC	palm kernel cake
PUFA	polyunsaturated fatty acids
QE	quercetin equivalent
qPCR	quantitative polymerase chain reaction
RFLP	restriction fragment length polymorphisms
rRNA	ribosomal ribonucleic acid
SAS	Statistical Analysis System
SFA	saturated fatty acids
SGLA	steroidal glycoalkaloid
SSF	solid-state fermentation
TAE	tris-acetate EDTA
TFC	flavonoid content
TPC	total phenolic content
UFA	unsaturated fatty acid

UV-Vis	ultraviolet to visible
VFA	volatile fatty acid

CHAPTER 1

INTRODUCTION

1.1 Introduction

Ruminant production in Malaysia is mainly aimed at fattening and subsequent slaughter (Zamri-Saad and Azhar, 2015). The animals are fed with agricultural by-products as feed resources (Predith et al., 2018). However, research to determine the cost-effectiveness of intensive feeding of ruminants is lacking (Mohamed et al., 2013). Similarly, besides palm oil by-products, studies on the processing of other agro-industrial by-products such as industrial potato waste (IPW) have not received special attention (Liew et al., 2017).

Potato is an important staple food crop that is consumed worldwide. It is ranked the fourth and fifth staple food crop in the world (Kareem et al., 2017). Processing of potatoes into finished products produces a million tonnes of waste, which is composed of starch, proteins, minerals and free amino acids (Li et al., 2011). Industrial potato waste (IPW) is a by-product from the potato processing plant, it contains peels (skin), pulp, flesh (tuber) and low-grade potatoes, and is a good source of fibre such as insoluble cellulose, lignin, hemicellulose and pectin (Al-Weshahy et al., 2013); the fibre content may reach 40g kg^{-1} (Curti et al., 2016), depending on the method of peeling. Low-grade and cull potatoes as well as industrial potato wastes could fed to ruminants, however, potatoes are rarely used in the poultry feed industry (Sepelev and Galoburda, 2015). The use of IPW in pig and poultry diets is limited because of the poisonous solanine and chaconine contents (Heo et al., 2014). Also, IPW fed directly to ruminants could seriously affect dry matter intake because of its high moisture content (Fiems et al., 2013). In addition, other factors that may limit feed intake and utilization of IPW include the presence of antinutritional factors (ANF) such as phytic acid, tannin, and oxalate contents (Kareem et al., 2017). Besides, alpha solanine and chaconine are two major glycoalkaloids (secondary metabolites) in potato. The two compounds are toxic to animals, humans, and many microorganisms when ingested in large quantities (Omayio et al., 2016).

Energy is a major nutrient in IPW, the high starch content makes it an alternative source of energy to cereal grains on an equal dry matter (DM) basis (Franco et al., 2021). The starch component of IPW could be highly resistant to digestive enzymes because of the presence of antinutritional factors (ANF) (Li et al., 2015). Hence, excessive starch escapes the rumen to cause digestive disorders in the lower intestinal tract (Moharrery et al., 2014). However, IPW contains about 3 g kg^{-1} fibre content, thus it could not be used as a complete substitute for forage. Likewise, the crude protein content of potato by-products is relatively low (12 – 25 g kg^{-1}), hence feeding it to ruminants will require additional protein supplementation (Raina et al., 2023). In general, culled and potato by-products (waste/peels/skin/pulp) have been used as animal feed (Franco et al., 2021; Ncobela et al., 2017) because the by-products have no competition with humans (Bakshi et al., 2016).

Scientists over the years have used fermentation to overcome nutrient, storage, and antinutritional limitations of potatoes (Adegunloye and Oparinde, 2017) via solid-state fermentation. Fermentation is a process where microorganisms grow and multiply by utilizing and converting carbohydrates to carbon (iv) oxide and lactic acid. During fermentation, a plant fibre that is highly resistant to digestive enzymes is broken down by enzymes secreted by microbial organisms, thus bound nutrients are released (Hur et al., 2014).

Beneficial microbes that are capable of breaking down substrates produce different kinds of products. Depending on the type of nutrient attacked, microorganisms can be classified into proteolytic, lipolytic, and fermentative (De Beni Arrigoni et al., 2016). However, few microbial species are specifically proteolytic, lipolytic, or fermentative. In general, most microbes exhibit different fermentation properties in varying environmental conditions (Brzozowska and Oprządek, 2013). Nevertheless, microorganisms are known to dominate one another in the above basic classifications (Wolin et al., 2011). For instance, the breakdown of protein by proteolytic organisms yields products that are putrid with rotten odours and flavours. Similarly, lipolytic microorganisms that break down fats give rise to rancid and fishy odours. However, substrate fermentation by fermentative organisms produces products that are not offensive to tastes (Sharma et al., 2020).

Additional benefits of fermentation (besides preservation and nutrient enrichment) include inhibitory controls (Swain et al., 2014). The end products of food fermentation (alcohols and lactic acids) suppress proteolytic and lipolytic microorganisms that may find their way into the substrate, hence the drop in pH (< 4.6) inhibits microbes that thrive well in alkaline medium (Chen et al., 2013) to grow. When microbes ferment substrate, they derive energy in the process and thus multiply in numbers. Fermented products may have low energy value because parts of the carbohydrate are converted to alcohol (Anal, 2019).

Fermentation processes are accompanied by temperature elevation (Bumbieris Junior et al., 2017). The energy released as heat represents a gross energy fraction of the original substrate. Fermented products may have higher protein, amino acids, and vitamin values than the unfermented substrate (Nkhata et al., 2018). This is achieved not only through the catabolic process of breaking down complex compounds but also by the anabolic process of synthesising several vitamins and other growth factors (Moe, 2013).

Milling and steaming or cooking of substrate are not enough to release bound nutrients (Ortiz et al., 2018), but certain beneficial microbes are known to break down indigestible cell walls via enzyme activities, and by mycelia penetration (Adeyemo et al., 2013). Microbial fermentation allows enzymatic splitting of beta 1,4-glycosidic bonds in cellulose, hemicellulose, and related polymers that are resistant to the digestive enzymes of simple stomach animals (Qiu et al., 2016). After fermentation, dead microbial cells and their metabolites enrich the substrate with protein, vitamins, amino acids, and antioxidants (Nkhata et al., 2018; Ortiz et al., 2018). Beneficial microbes that are commonly used for fermentation in food industries include bacterial inoculants, fungi, and yeast (Joshi et al., 2015).

1.2 Problem Statement

Industrial potato waste (IPW) is substantially produced by potato processing plants in Malaysia. The term IPW is synonymous to potato waste, potato processing waste, potato peels, potato by-product, potato residue and industrial potato peels (Franco et al., 2021; Ncobela et al., 2017; Sepelev and Galoburda, 2015; Hamed et al., 2011; Tavares et al., 2011; Onwubuemeli et al., 1985 and Tawila et al., 2008). In the present study, IPW refers to the by-products from potato processing plants. Depending on the processing method and variety of potatoes, potato waste can range between 15% to 40% of the original fresh weight (Sepelev and Galoburda, 2015). Although local production of potatoes stands at 215,632.40 t in 2022 with a potential yield of 8.3 t/ha (Department of Agriculture, 2022); Malaysia imported 217,636.81 t of potato in 2021 (FAOSTAT, 2023). The combined volumes of potatoes (local and import) could generate 64,99.38 to 173,307.68 t of waste (peels/skin/low grades and rejected potatoes) from processing plants, households and restaurants. Considering the production of finished products with concomitant waste generation of local potato processing plants such as French Fries (Malaysia) Sdn. Bhd, the IPW could be used as a potential animal feed. Although one of the limitation of IPW is high moisture content (85 – 87 %), which will make the cost of transportation from the processing plant to animal farms exorbitantly higher due to the fact that bulk of the weight is moisture instead of dry matter; the challenge could be solved by further processing the waste to moisture level of less than 10 %.

The processing of potatoes into finished products produced industrial waste that is primarily composed of potato peels, tuber portions and low-grade products (see Appendix A). The higher composition of peels in the IPW virtually increased the fibre content, and antinutritional factors (ANF) especially glycoalkaloid compounds. More so, the peels have a higher concentration of phenolic compounds than the tuber fraction. Nevertheless, few studies have been conducted on IPW as a potential feed material for ruminants.

Although, IPW could be stored fresh for a couple of months under controlled temperatures with good ventilation; such process is economically impractical. Alternatively, IPW could be preserved for a longer period by ensiling either alone or in a mixture of straws or grasses. On the other hand, IPW could be processed by short-term fermentation via a solid-state fermentation method which last for a couple of days (< 7 days). The major limitation of the longer fermentation method is the duration of time (≥ 21 days) before the substrate reaches a stable anaerobic condition.

Notwithstanding, several studies were previously conducted on fermenting potato waste using microbial species such as *Saccharomyces cerevisiae*, *Aspergillus niger*, *Streptococcus thermophilus*, and *Bacillus subtilis* via a solid-state fermentation. A short-term fermentation provides an opportunity to quickly improve the nutritive values of a substrate within a few days (24-96 hours). Afterwards, the substrate is subsequently dried to ensure a longer shelf-life. From available literature, studies conducted on the fermentation of potato wastes via solid-state fermentation were mostly related to pharmaceuticals, breweries, enzyme assays, and proximate constituents (Waseem Ali et al., 2017). Besides, there is little or no work that was conducted to compare the fermentation potentials of beneficial species of bacteria, yeast and fungi. In addition, there are virtually limited works on the fermentation of industrial potato waste related to ruminant nutrition. Hence, three species of *Lactiplantibacillus plantarum, S. cerevisiae,* and *Aspergillus oryzae* were purposely selected for the study because of their record of effectiveness and widely used in the food industry.

The present study aims to evaluate the effects of fermenting industrial potato waste with *L. plantarum, S. cerevisiae,* and *A. oryzae* via the solid-state fermentation (SSF) method. Thus, phenolic compounds, antinutritional factors, antioxidant capacity, and *in vitro* digestibility as well as ruminal fermentation characteristics will be evaluated.

1.3 Hypothesis statement

Fermenting industrial potato waste using *L. plantarum*, *S. cerevisiae* and *A. oryzae* improves nutrient contents, phenolic compounds, antioxidant capacity, *in vitro* digestibility, and reduces methane gas production as well as glycoalkaloid compounds better than the uninoculated (control) treatment.

1.4 Objectives

The study aims to evaluate industrial potato waste fermented with *Lactiplantibacillus plantarum, Saccharomyces cerevisiae*, and *Aspergillus oryzae* on nutritive contents, phenolic compounds, antioxidant capacity, *in vitro* digestibility, and fermentation characteristics.

However, the specific objectives of the study were:

1. To validate and characterize isolates of *Lactiplantibacillus* spp., *Saccharomyces* spp., and *Aspergillus* spp.

2. To determine gross energy, starch components, proximate and fibre composition of industrial potato waste fermented with *L. plantarum, S.* cerevisiae, and *A. oryzae.*

3. To determine phenolic compounds, antioxidant capacity, and glycoalkaloid contents of industrial potato waste fermented with *L. plantarum, S.* cerevisiae, and *A. oryzae.*

4. To determine *in vitro* digestibility, metabolizable energy, microbial population, and rumen metabolites of industrial potato waste fermented with *L. plantarum, S.* cerevisiae, and *A. oryzae.*

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