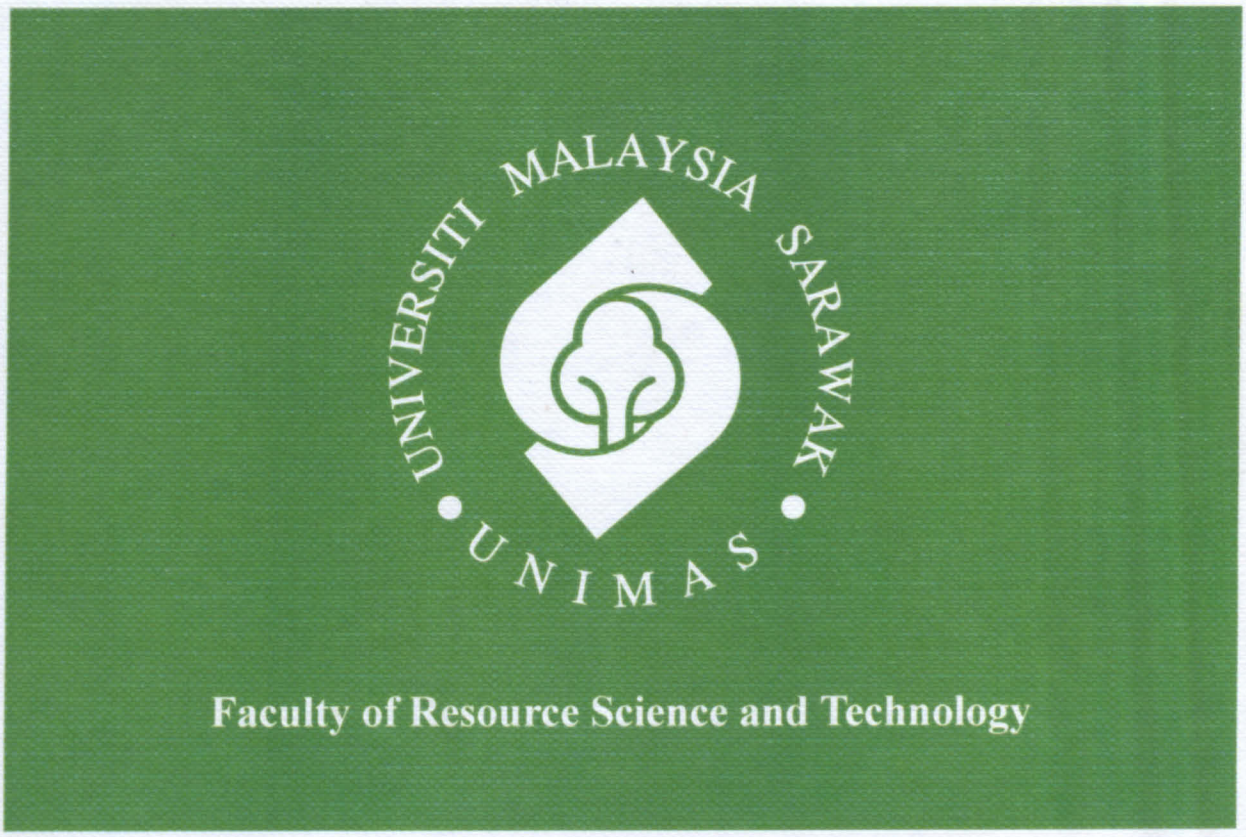

Effects of Yeast Inoculum Sizes on Fermentation of *Petai Belalang* Seeds for Coffee-like Beverage Production

Nurul Nadiah Binti Ahmad Daud

(50876)

Bachelor of Science with Honours
(Resource Biotechnology)
2018

Effects of Yeast Inoculum Sizes on Fermentation of *Petai Belalang* Seeds for Coffee-like Beverage Production

Nurul Nadiah Binti Ahmad Daud (50876)

A Thesis Submitted in Partial Fulfilment of the Requirement for the Degree of Bachelor of Science with Honours (Resource Biotechnology Programme)

Supervisor: Dayang Salwani Binti Awang Adeni, Dr

Resource Biotechnology Programme

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

2018

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Final Year Project Report ☑

Masters ☐

PhD ☐

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I, **Nurul Nadiah Binti Ahmad Daud (50876)** from **Faculty of Resource Science and Technology** hereby declare that the work entitled **Effects of Yeast Inoculum Sizes on Fermentation of *Petai Belalang* Seeds for Coffee-like Beverage Production** is based on my original work, except for the quotations and citations which have been dully acknowledged also, declare that it has not been or concurrently submitted for any other degree at UNIMAS or other institution of higher learning.

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ABSTRACT

This study focused on *Saccharomyces cerevisiae* inoculated in different inoculum sizes to investigate which inoculum size is the best for producing the highest starch containing high reducing sugars in optimum time of fermentation. In this experiment, *S. cerevisiae* or yeast was inoculated from the cultured colony and prepared as five different inoculum sizes in (%v/v); 5%, 10%, 15%, 20%, and 25%. Each inoculum was standardized with 15 ml distilled water and 5 g of mimosine-reduced *Petai Belalang* seeds. For both analysis of starch and protein content, batches without the addition of yeast acted as positive control. Protein content was analysed in relation of production of starch content. The highest starch content was produced by 15% yeast inoculum at 18 h. Next, the content of reducing sugars of fermented, mimosine-reduced *Petai Belalang* seeds were further analysed using DNS analysis. Before that, the highest and lowest content of starch (based on starch analysis) for each yeast inoculum (%v/v) from 5%, 10%, 15%, and 20% were determined. The result exhibited the highest reducing sugar contents was yielded by replicate labelled C at 18 h of 15% yeast inoculum. Therefore, the replicate was chosen as the best pick to be used for further study of mimosine-reduced *Petai Belalang* seeds roasting process and were compared with the non-fermented, dried *Petai Belalang* seeds in terms of colour and aroma.

Keywords: *Saccharomyces cerevisiae*, Inoculum sizes, starch analysis, protein analysis, biomass

KESAN SAIZ INOKULUM YIS TERHADAP FERMENTASI BIJI *PETAI BELALANG* UNTUK PENGHASILAN MINUMAN BERCIRIKAN KOPI

ABSTRAK

Kajian ini menfokuskan inokulasi Saccharomyces cerevisiae ke dalam setiap saiz inokulum untuk mengenal pasti saiz inokulum yang terbaik untuk menghasilkan kandungan kanji yang tertinggi yang mengandungi gula reduksi dalam masa fermentasi yang optima. Dalam eksperimen ini, S. cerevisiae ataupun yis telah diinokulasikan daripada koloni yang dikultur dan disediakan sebagai lima saiz inokula yang berbeza (%v/v); 5%, 10%, 15%, 20%, dan 25%. Setiap inokulum diselaraskan dengan 15 ml air distil dan 5 g biji Petai Belalang yang kurang mimosine. Untuk kedua-dua analisis kandungan kanji dan protein, set tanpa penambahan yis memainkan peranan sebagai kontrol positif. Kandungan protein dianalisis telah berkait hubung dengan penghasilan kandungan kanji. Kandungan kanji yang tertinggi telah dihasilkan oleh 15% inokulum yis pada jam ke-18. Seterusnya, kandungan gula reduksi dalam biji Petai Belalang yang kurang mimosin dan difermentasi menjalani analisis DNS. Sebelum itu, kandungan kanji yang tertinggi dan terendah (berdasarkan analisis kanji) untuk setiap inokulum yis (%v/v) daripada 5%, 10%, 15%, dan 20% telah dikenal pasti. Keputusan yang diperoleh menyatakan bahawa kandungan gula reduksi tertinggi telah dihasilkan oleh replika C yang ke-18 jam daripada 15% inokulum saiz. Oleh itu, replika terbaik ini telah menjalani fermentasi sekali lagi untuk tujuan pemanggangan dan dibandingkan dengan biji Petai Belalang yang hanya dikeringkan tanpa menjalani fermentasi dari segi warna dan aroma yang terhasil.

Kata kunci: *Saccharomyces cerevisiae, saiz inokulum, analisis kanji, analisis protein, biomas*

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°C	Temperature
%	Percentage
kg	Kilogram
g	Gram
mg	Milligram
mm	Millimetre
nm	Nanometre
ml	Millilitre
g/16 g N	Gram per 16 gram Nitrogen
g/kg	Gram per kilogram
mg/100 g	Milligram per 100 gram
g/L	Gram per litre
kcal/kg	Kilocalorie per kilogram
mg/ml	Milligram per millimetre
% v/v	Percentage volume per volume
ME	Metabolizable Energy
EE	Ether extract
NFE	Nitrogen Free Extract
CF	Crude fibre
N	Nitrogen
rpm	*Rotation per minute*
M	*Molar*
$	*Dollar*
h	*Hour*
min	*Minute*

1.0. INTRODUCTION

1.1. Research Background

Coffee is a worldwide trade commodity and well-known to be one of the most consumed beverages in the world. Coffee processing has been an art with scientific interest to understand the many physical and chemical mechanisms involved. Additionally, there has been a surge in demand for specialty coffee from different origins and roasted differently.

The need to find an alternative to produce coffee with quality enhancement which is cost effective has become imperative. The common industrial process of coffee production utilises the raw coffee cherries. This study is an approach to find alternative in non-conventional materials form which can be easily obtained and is economically beneficial especially to the rural communities.

Leucaena is a genus of flowering plants classified into subfamily *Mimosoideae,* family *Fabaceae* with approximately 22 species of shrubs and trees which are generally known as lead trees. *Leucaena Leucocephala* (*L. leucocephala*) or locally known as *Petai Belalang* belongs to this family is a small, fast growing mimosoid tree native to northern Central America and southern Mexico but has now distributed throughout tropics (Brewbaker, & Sorensson, 1990). *Petai belalang* was referred to as the "miracle tree" because of its multipurpose nature and medicinal efficacy (Abdelhady & Abdallah, 2017).

As mentioned by Hui *et al.* (2010), fermentation has potential in producing natural flavours. With a global industrial size estimated at US$ 16 billion in 2003, all compounds that produce aroma and fragrance have been widely applied in commercial products such as food, cosmetics, detergents, feed, and pharmaceutical industries (Serral *et al.*, 2005). By

fermentation, yield of natural aroma compounds allows the recovery of natural food additives which is preferred by the consumers.

Particularly for flavour preparation, many species of yeast have been utilized. The most commonly used microorganism in organic syntheses is *Saccharomyces cerevisiae* (*S. cerevisiae)* because it is cost-effective, versatile, and can be easily obtained. *S. cerevisiae* are grown commercially as it plays a crucial role in food and beverage industries. According to Evangelista *et al.* (2014), coffee with special aroma can be improved by yeast starters, specifically *S. cerevisiae* during fermentation.

1.2. Problem Statement

In natural fermentation, problem such as lack of control occurs leading to contamination risk and quality decrement (Dierings *et al.*, 2013). Commercial yeast is utilized to better control the fermentation and reduces the risk of negative organoleptic impacts due to the growth and metabolism of other indigenous yeasts (Cabranes & Mangas, 1997; Valles *et al.*, 2008). This study covered the induced fermentation on *Petai Belalang* seeds using selected yeast strain to determine the best yeast inoculum size for further study in the future.

1.3. Objectives

The objectives in initiating the research is:

1. To investigate the effect of yeast inoculum sizes on fermentation of mimosine-reduced *Petai Belalang* seeds for coffee-like beverage.
2. To investigate the inoculum size that determines the highest reducing sugar content to produce coffee-like aroma via roasting.

2.0. LITERATURE REVIEW

2.1. Importance of Coffee in World Trade

Coffee is an important commodity in the world economy, which represents for trade worth roughly US$ 16.5 billion when some 97 million bags of 60 kg (5.8 million tons) were shipped in 2010 (International Trade Centre, 2011). Exploitation of *Coffea Arabica* seeds for commercial production accounts for roughly 70% of the world market while *Coffea Canephora* represents about 30% (Crozier *et al.*, 2012). For many countries, coffee exports are crucial for foreign exchange earnings, proportion of tax income, and gross domestic products.

2.1.1. Coffee Production in Malaysia

Coffee has been identified as one of the potential commodities by the government in the third National Agricultural Policy (NAP3, 1998-2010). This commodity has crucial role in the socioeconomic development and the welfare of the rural communities. Coffee enterprise is conducted at minute scale, mostly cultivated by small farm holders due to competition with other industrial plants, less favourable returns, unstable prices, as well as the complexities of harvesting and processing (Nor & Wahab, 2016).

2.1.2. Coffee Processing

Every step of coffee processing includes coffee plant selection, proper harvesting of the beans for each processing method, processing of beans, drying, hulling, roasting, grinding, and cupping are important to maintain a high-quality coffee (Bee *et al.*, 2005). Apparently, exocarp, mesocarp, and mucilage layer are removed during processing as coffee is prepared from the pair of seeds located centrally within the coffee cherry. Lee *et al.* (2015) reported that mucilage layer locates beneath the mesocarp which is colourless and viscous.

2.1.2.1. Coffee Roasting

According to Adriana Farah (2012), the complex series of physical and chemical changes in the seeds are caused by high roasting temperature and the specific roasting conditions consequently affect the bioactivity and flavour of the beverage. Physically, changes such as weight loss, colour, and cracking sound can be investigated (Fareez Edzuan *et al.*, 2015). The coffee beans also undergo the chemical changes of main components such as oligosaccharides, trigonelline, proteins, peptides and free amino acids, as well as formation of aroma components and melanoidins during roasting process (Wei & Tanokura, 2015).

2.2. *Petai Belalang* Seeds

The raw seeds have a hard texture and ovoid shape. They have brown hulls and yellow kernels. As clarified by Orwa *et al.* (2009), these seeds have a hard, shiny testa with 6.7-9.6 mm long and 4.0-6.3 mm wide which aligned transversely in the pod. Legumes can be directly served as human food. The roasted seeds are substituted as coffee and the young pods are served as vegetable in Philippine Islands (Zayed *et al.*, 2018). According to Zayed and Benedict (2016), just like any other leguminous seeds, *Petai Belalang* seeds are considered as non-conventional sources of protein.

2.2.1. Chemical composition and nutritive value of *Petai Belalang* Seeds

Protein qualities and metabolizable energy values are quite variable while the analysed total amino acids and energy contents of many grain legumes are quite similar (Ahmed & Abdelati, 2009). As mentioned by Alabi and Alausa (2006), *Petai Belalang* seeds contain the highest amount of carbohydrates, crude proteins, lipids, and ash contents compared to *Lablab purpureus* and *Mucuna Utilis.* Apart from that, Bakti (2003) stated that the seeds contain similar amount of protein to soy beans.

Besides, the composition of *Petai Belalang* seeds in content of crude proteins, amino acids profile, and total phosphorus is lower than soybean meal whereas it is higher in ME, EE, and CF (National Research Council, 1994). Apparently, *Petai Belalang* seeds contained a high composition of protein which is 31.3% (Ahmed & Abdelati, 2009). However, nutritional values of the seeds are varied depending on geographical location and cultivars.

Apart from that, trigonelline is an alkaloid detected in the seeds, pods, leaves, and flowers but not in the roots (Ogita *et al.*, 2014). It contributes to bitterness which serves to produce important aroma compounds. As mentioned by Abdelhady and Abdallah (2016), the seeds contain flavonoids such as caffeic acid, gallic acid, quercetin, kaempferol, and luteolin.

The detailed chemical composition and amino acids composition of *Petai Belalang* seeds are shown in **Table 1** and **Table 2** respectively (Ahmed & Abdelati, 2009).

Table 1. Chemical composition of *Petai Belalang* seeds.

Item	Analysis
ME	2573.26±4.24 kcal/kg
Crude protein	311.00±3.61 g/kg
Crude fat	56.00±4.0 g/kg
Crude fiber	132.00±2.0 g/kg
Dry matter	948.00±1.0 g/kg
Crude ash	45.00±5.0 g/kg
NFE	404.00±2.0 g/kg
Calcium	3.70±0.1 g/kg
Total Phosphorus	3.40±0.001 g/kg
Tannin%	0.75±0.02
Phytate mg/100 g	697.50±1.5

Table 2. Amino acids composition of *Petai Belalang* seeds.

Amino acids	g/kg	g/16 g N
Cystine	3.50±0.1	1.13
Arginine	26.20±2.0	8.42
Methionine	3.60±0.05	1.16
Glutamic acid	46.30±0.27	14.89
Threonine	8.70±0.1	2.80
Glycine	13.80±0.1	4.44
Alanine	11.10±0.1	3.57
Valine	11.10±0.2	3.57
Isoleucine	9.30±0.3	2.99
Leucine	18.10±0.3	5.82
Lysine	13.90±0.2	4.47
Methionine + Cystine	7.10±0.02	2.28

2.3. Application of Fermentation

The two types of fermentation are alcoholic and lactic acid which occurred by the action of yeast and bacteria respectively. Mass culture of microorganisms such as yeasts, bacteria, and moulds which aid in converting raw materials into organic products (Stanbury *et al.*, 2017). These microorganisms may be added to the raw materials as starter cultures or are part of the microorganism ecosystem of the raw materials.

2.3.1. Fermentation Medium

As reported by Singh *et al.* (2016), the most suitable fermentation conditions and the necessary medium components must be determined and optimized appropriately. Fermentation conditions include temperature and agitation speed, whereas medium components are carbon and nitrogen sources. By further optimizing these parameters, maximum concentration of desired products could be achieved (Franco-Lara *et al.*, 2006; Wang *et al.*, 2011)

2.3.2. Microbiology of Fermentation

Microbial fermentations apply a series of complex process through which substrates (typically food) are converted into products due to the growth and metabolic activities of endogenous and exogenous microorganisms (Batt, 2016). Sometimes, the process requires the involvement of one substrate component and one microorganism, but some fermentations need a complex mixture of substrates and several microorganisms.

2.3.3. *Saccharomyces cerevisiae* (*S. cerevisiae*) as Starter Culture

Starter cultures assist in improving the quality of fermented food by providing predictability of the final product and better fermentation control (Evangelista *et al.*, 2014). Silva *et al.* (2013) stated that yeast *S. cerevisiae* or yeast has a good potential to be used as starter cultures for *Petai Belalang* seeds fermentation. Enzymes secretion of *S. cerevisiae* hydrolyses the seeds' mucilage, thus, improving the quality of the fermentation process.

3.0. METHODOLOGY

3.1. Overview of Methodology

Figure 1 shows the overview of the methodology to study the effects of yeast inoculum sizes on fermentation of mimosine-reduced *Petai Belalang* seeds for coffee-like beverage.

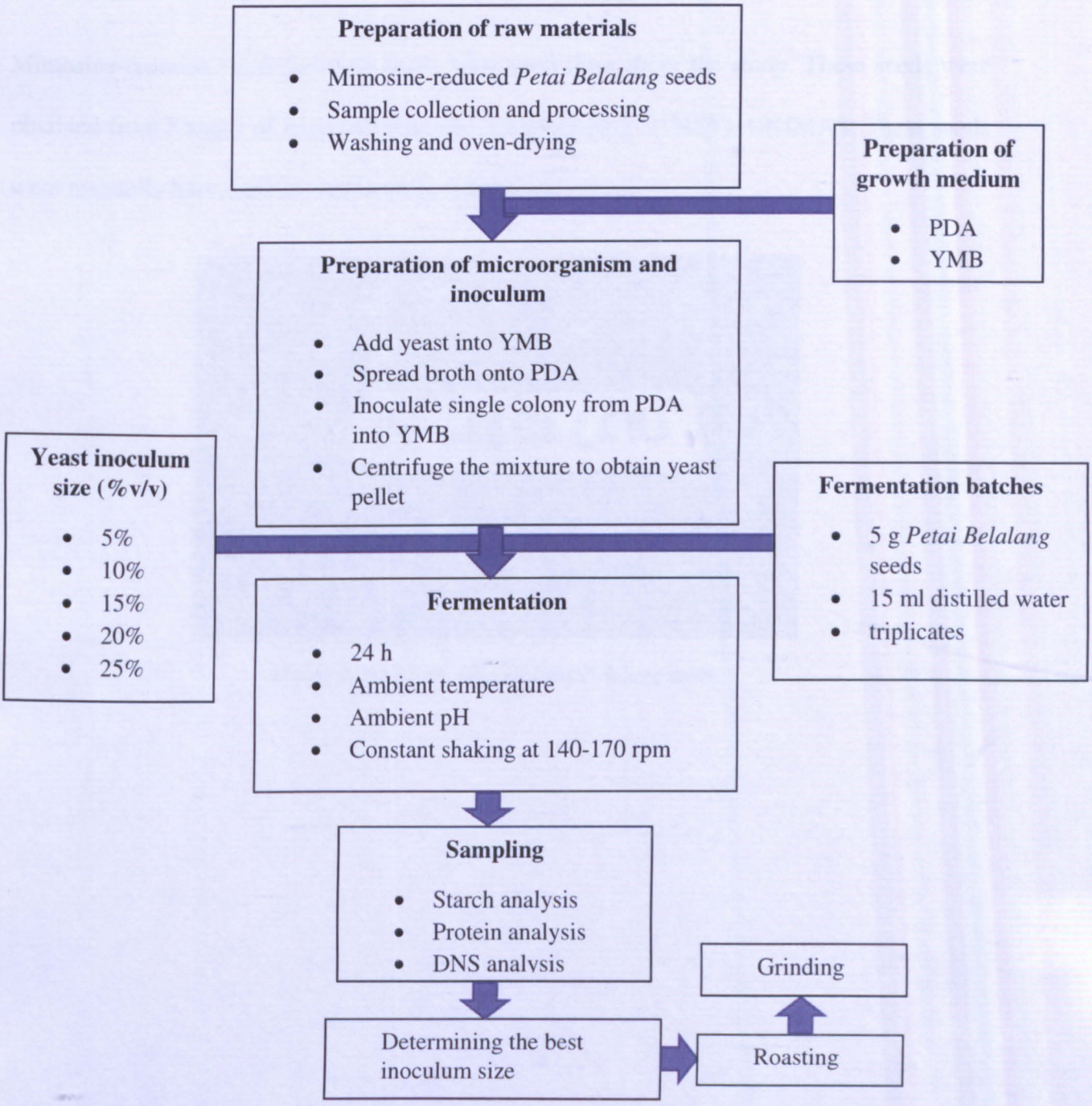

Figure1. Flowchart of methodology throughout the project.

3.2. Materials

3.2.1. Microorganism

S. cerevisiae or commonly known as commercialised yeast was purchased from local market that supplied by Mauripan Baking Industry, Australia.

3.2.2. Raw Materials

Mimosine-reduced *Petai Belalang* seeds were used throughout the study. These seeds were obtained from Faculty of Resource Science and Technology (FRST), UNIMAS. These seeds were manually harvested at mature stage (**Figure 2**).

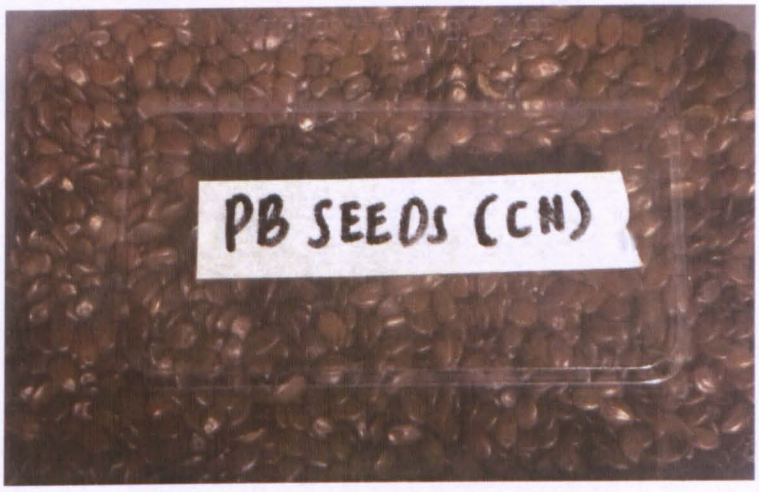

Figure 2. Mimosine-reduced *Petai Belalang* seeds.

3.2.3. Inoculum

In this study, five different inoculum sizes were used as the parameter to be investigated. The range of inocula used was 5-25% in term of (v/v%). The unit (%v/v) was used because the preparation of fermentation batches involves working volume (liquid) and inoculum (liquid). Based on the **Equation 1**, calculation of the inocula (V_{solute}) can be done easily.

$$\frac{v}{v}\% = \frac{\text{Volume of inoculum (ml)}}{Volume\ of\ \text{inoculum + media}\ (ml)} \times 100\%$$

Equation 1. Equation of (%v/v) of yeast inoculum sizes.

Where,

V_{solute} = inoculum

$V_{solution}$ = Working volume (inoculum + media)

Fermentation media (working volume) used was 15 ml distilled water and desired range of inoculum sizes was known. However, the concentration of inocula to be used was unknown. Therefore, the above equation (**Equation 1**) was used to determine the concentration of yeast (%v/v) per inoculum sizes. Prior to the different percentage of inoculum sizes, each centrifuge tube was filled with 0.75 ml yeast solution as the standard volume (**Table 3**). Additionally, the calculation to determine the volume of each inoculum was based on 15 ml of distilled water (**APPENDIX 1**).

Table 3. Diluted yeast solution needed for each inoculum size.

Inoculum Size, %	**Volume of Yeast Pellet, ml**
5	0.75
10	1.50
15	2.25
20	3.00
25	3.75

3.2.4. Control

Mimosine-reduced *Petai Belalang* seeds acted as control in the fermentation process of pre-treated, fermented mimosine-reduced *Petai Belalang* seeds. The non-treated batches were not added with yeast inoculum as contrast to the treated batches.