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THE ACTIVITY OF BETA-GLUCOSIDASE TOWARDS THE PRODUCTION OF FARNESOL IN JOSAPINE WASTE

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DISSERTATION SUMMITED IN PARTIAL FULFILMENT OF THE
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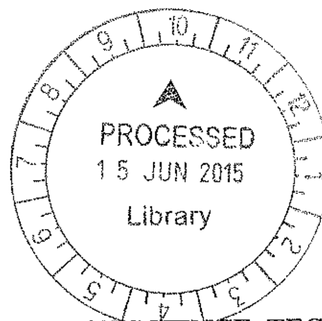

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ABSTRACT

Terpenes have its proven protective functions but recently researchers have found that farnesol has anti-cancer agent. The fermentation of Josapine waste using *Saccharomyces cerevisiae* that comprises beta (β)-glucosidase might enhance total content of farnesol which is useful in the biopharmaceutical industry. The aims of this study were to compare and quantify the β-glucosidase activity in the fermented peel and pulp of Josapine as well as to relate the amount of β-glucosidase to farnesol extracted from the fermented peel and pulp. *S. cerevisiae* was able to ferment the pulp and peel of Josapine to yield farnesol. Fermentation was carried out for 0 hour, 24 hours and 48 hours separately for both natural and microbial fermentation. The cell density and pH of the fermented broths were recorded with intervals of 0 hour, 24hours and 48 hours respectively. Farnesol was extracted from the fermentation broths by using hexane as the organic solvent. Farnesol separation was carried out by using adsorption chromatography. The farnesol obtained from the separation was quantified using UV-visible spectrophotometer at 290 nm. However, the amount of farnesol was relatively low may be due to the inefficiency of *S. cerevisiae* in using the existing substrate(s) in the pulp and peel of Josapine. It is expected farnesol was not eluted out properly during the separation.

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LIST OF ABBREVIATIONS

%	percentage
β	beta
°C	degree Celsius
LAF	laminar air flow
MeOH	methanol
Rpm	revolutions per minute
SPE	solid phase extraction
UV	ultraviolet
GRAS	Generally Recognized As Safe
pNPG	p-nitrophenyl-ß-D glucopyranoside
MARDI	Malaysian Agricultural Research and Development Institute
MS	mean square
NCBI	National Centre for Biotechnology Information
w/v	weight/volume
v/v	volume/volume
PBS	phosphate buffered saline
pH	power of hydrogen
pNP	p-nitrophenol
P value	a probability that ranged from zero to one
<	Greater than
>	Less than
YPD	yeast extracts peptone dextrose
α	Significant level
SS	sum of square
mg	milligram
g	gram

OD_{600}	Optical density 600nm
mL	milliliter
min	minutes
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
CoA	Coenzyme A
n=3	Number of replicates

CHAPTER 1

INTRODUCTION

There are various types of pineapple species that are being grown in Malaysia. Such types include Josapine, Sarawak, N 36, Morris, Spanish, Queen and several more (Chan & Lee, 1996). Malaysia is one of the leading countries that produce tonnes of pineapple that are waiting to be canned, jammed and juiced. The canned pineapple industry that has a market network of 32 countries worldwide has helped increase the pineapple production in Malaysia. However, this also simultaneously produces countless amount of pineapple waste such as peels and pulps. These solid waste generated can be put into good use such as cellulose production by some fungi cultured on pineapple waste (Omojasola *et al.*, n.d.), vinegar production (Roda *et al.,* 2014), and farnesol extraction from the fermented juice of Josapine (Kameel, 2013). In this research, the extraction of farnesol present in the peels and pulps became the center of this research.

Farnesol is a colorless sesquiterpenes based alcohol (Wang *et al.*, 2010). The natural extraction of farnesol from plants, animals and sometimes from microorganisms is usually inefficient, therefore synthetic farnesol is produced. Nonetheless, the production cost of this synthetic farnesol is high due to the complicated processes involved in producing them. In addition, now more efforts are given in engineering *Escherichia coli* and yeast culture to produce farnesol and sesquiterpenes (Wang *et al.*, 2010). Conversely, this process is also very costly and has very low stability and less productive in industrial scales production.

With wide knowledge on the fermentation technology, industrial researchers have looked into fermentation as a source for the essential oil production. Engineered *E. coli* and yeast culture have been practiced in the industrial production of farnesol and also reported that its successful rate was high. However, engineering the *E. coli* and yeast culture are very costly and not very effective at the industrial scale due to instability

(Wang *et al.*, 2010; Millis *et al.*, 2004). For the research purposes, we had opted for non-engineered yeast cells (*Saccharomyces cerevisiae).* It is regularly used in the fermentation due to the Generally Recognized As Safe (GRAS) microorganism. In addition, it also can grow in large number in a shorter time and it is non-fastidious microorganism. Hence, the purpose of this project was to investigate the possibility of non-engineered *S. cerevisiae* to produce farnesol using fermentation technology.

The main objectives of this project are as follows:

- to compare the beta (β) -glucosidase activity of indigenous yeasts, and to determine their role in the production of flavours after the fermentation of peels and pulps of Josapine.
- to quantify β-glucosidase and relate its amount to the content of farnesol present in the fermented peels and pulps of Josapine.
- to carry out two types of fermentation which are natural fermentation and yeast fermentation of peels and pulps of Josapine.

CHAPTER 2

LITERATURE REVIEW

2.1 TERPENE

There are many types of terpenes such as monoterpene, sesquiterpene, hemiterpenes and many more. Sesquiterpene, a 15-carbon compound is discovered principally in higher plants, and some invertebrates. Due to the expanded chain length and extra double bond found in the acyclic sesquiterpenes, therefore it is the most diverse functional group of isoprenoids and also the main constituent of essential oils in plants. In plants, they work as pheromones and juvenile hormones. Some of common sesquiterpenoids are demonstrated in Figure 2.1. The non-cyclic delegate are additionally called farnesans, term obtained from the fundamental structure, farnesol. Farnesol and nerolidol are exceptionally regular and are confined from crucial oils of different sources.

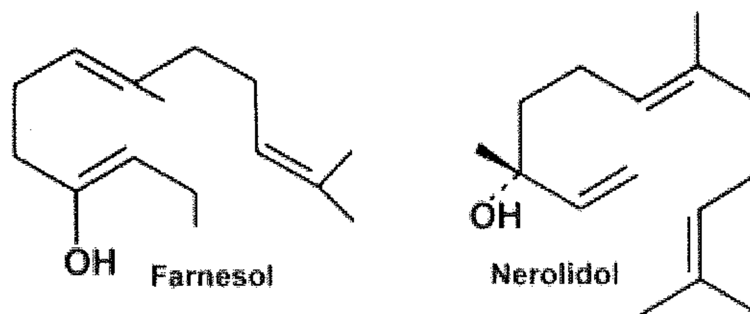

Figure 2.1 The structure of farnesol and nerolidol

2.2 FARNESOL

Farnesol is acyclic volatile sesquiterpene alcohol produced from five-carbon isoprene compound from both animal and plant. Mostly, this alcohol compound always remains colorless under standard conditions (National Centre for Biotechnology Information (NCBI), n.d). However, it has high solubility in alcohol but insoluble in water because of its hydrophobic condition and it react miscible (mix evenly without forming any layer separating the solutions) when placed with oil. Farnesol is one of the most used compounds in the deodorant, cosmetic, perfume, pharmaceutical and in some industrial materials (Wang, 2011; NCBI, n.d).

In addition, this sesquiterpene is also used as natural pesticide to chase away the mites and several insects. Moreover, in some cases they even use farnesol for attracting mate purposes in elephants and bulls. The most interesting part is, some spiders built their webs with the presence of farnesyl acetate so that they can attract the female spiders for mating (Cugini *et al.*, 2007).

The production of farnesol using the plant, animal and sometimes from microbial is very insufficient. The synthetic farnesol was very costly due to the challenging process involved. Moreover, farnesol was likewise shown to be the "quorum-sensing molecule" distinguished in fungi (Hornby *et al.*, 2001). The vicinity of farnesol keeps the yeast-to-mycelium transformation, bringing about effectively budding of yeasts without impacting cell growth rates. This study is the first to distinguish an extracellular molecule intervening an eukaryotic quorum-sensing system (Hornby *et al.*, 2001).

2.3 BETA (β)-GLUCOSIDASE.

β-glucosidase is one of the cellulases, it decompose the cellulose and related polysaccharides to simpler form to produce energy (Ferreira, n.d). It hydrolyses the terminal non-reducing residues in β-D-glucosides by releasing glucoses as shown in Equation 2.1.

β-D-glucoside + H_2O $\xrightarrow{\beta\text{-glucosidase}}$ D-glucose + Alcohol (Equation 2.1)

This enzyme is the important ingredient for degradation of plant cell walls by pathogens. The aroma compounds in pineapple are mostly terpenes, which are metabolites derived from mevalonic acid. In recent years, it has been found that a considerable portion of these compounds occurs in bound forms, particularly glycosides, which do not seem to contribute to the aroma unless they are hydrolysed. The glycosidically-bound forms can be converted into the free odourous forms such as the linalool, terpineol, farnesol and citronellol and many more, by hydrolysis with glycosidases (Bayonove *et al.*, 1992; Aryan *et al.*, 1987; Biron *et al.*, 1988; Gunata *et al.*, 1990). The hydrolysis of farnesol-glucoside by β-glucosidase is shown in Equation 2.2.

Farnesol-glucoside $\xrightarrow{\beta\text{-glucosidase}}$ Glucose + Farnesol (Equation 2.2)

These compounds can be found in pineapple and musts as free, volatile and odorous forms as well as in flavourless, nonvolatile forms β-glycosidically bound to disaccharide molecules (Vasserot *et al.*, 1990). These bound flavourless glycosidic complexes are generally more abundant than free odorous forms and they represent a potential source of the fragrant compounds in pineapple requiring enzymatic or acidic hydrolysis for the liberation of their fragrances (Gunata *et al.*, 1990).

2.4 Mevalonate Pathway

Mevalonate pathway is an important cellular metabolic pathway present in all higher eukaryotes and many bacteria. While broadly examined in respect with cholesterol synthesis and its implicative suggestions in cardiovascular disease, lately the mevalonate pathway has turned into an interesting theme, when an enormously huge number of trial and clinical studies proposed that restraint of non-sterol isoprenoids may have significant enthusiasm for human pathology (Wang, 2010). Figure 2.2, shows the mevalonate pathway in which, statins is used to reduce the cholesterol level and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) is formed by condensation of acetyl-CoA and acetoacetyl-CoA, catalyzed by HMG-CoA synthase. As the process continues, it is proven to produce farnesol as shown Figure 2.2.