# BETA-GLUCOSIDASE ACTIVITY AND LINALOOL PRODUCTION IN THE FERMENTATION OF PINEAPPLE WASTE

MICHELLE POH SIN YI

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Name

I1200 1726

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## ABSTRACT

Linalool is one of the most interesting acyclic terpene alcohols, both from the point of view of academic interest and of practical value. Studies have shown that some essential oils which contain linalool have antimicrobial, antibacterial and antiviral effects. In this study, the production of linalool by fermentation of the pulp and peel of pineapple Josapine using *Saccharomyces cerevisiae* was tested. Fermentation was carried out for 24 hours and 48 hours separately for both microbial and natural fermentation. The cell density and pH of the fermented broths were recorded with intervals of 0, 24 and 48 hour(s) respectively. Linalool was extracted from the fermentation broths by using dichloromethane as the organic solvent. Linalool separation was carried out by using adsorption chromatography. The linalool obtained from the separation was quantified using UV-visible spectrophotometer at 300 nm. Based on the results, the activity of β-glucosidase showed an increase in activity, however, the yield of linalool produced through both microbial and natural fermentations of pulp and peel did not show any significant differences from 0 hour to 48 hours of fermentation. Although linalool was successfully extracted, the amount did not reflect β-glucosidase activity measured in both fermentations.

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| | |
|---|---|
| % | percentage |
| $<$ | less than |
| $\leq$ | less than or equal to |
| $=$ | equal |
| µL | microliter |
| ANOVA | analysis of variance |
| $A_{600}$ | absorbance at 400 nm |
| $C_5$ | five carbons |
| $C_5H_8$ | isoprene |
| cells/mL | cells/millilitre |
| cm | centimeter |
| df | degrees of freedom |
| DMAPP | dimethylallyl diphosphate |
| DNA | deoxyribonucleic acid |
| F value | Fisher's value |
| $F_{crit}$ | F critical value |
| g | gram |
| IPP | isopentyl diphosphate |
| IUPAC | International Union of Pure and Applied Chemistry |
| LAF | laminar air flow |
| MARDI | Malaysian Agriculture Research and Development Institute |
| MEP | 2-c-methyl-D-erythritol-4-phosphate |

| | |
|---|---|
| mg | milligram |
| mg/dL | milligram/decilitre |
| mL | millilitre |
| MPIB | Malaysian Pineapple Industry Board |
| MS | mean square |
| MTS | monoterpene synthase |
| MVA | mevalonic acid |
| n=2 | duplicates |
| n=3 | triplicates |
| $Na_2CO_3$ | sodium carbonate |
| NaCl | sodium chloride |
| NaOCl | sodium hypochlorite |
| nm | nanometer |
| °C | degrees Celsius |
| $OD_{600}$ | optimal density at 600 nm |
| P value | a probability with a value ranging from zero to 1 |
| PBS | phosphate buffered saline |
| pH | potential of hydrogen |
| pNP | p-nitrophenol |
| pNPG | p-nitrophenyl-β-D-glucopyranoside |
| rpm | revolutions per minute |
| *S. cerevisiae* | *Saccharomyces cerevisiae* |
| SS | sum of squares |

| | |
|---|---|
| UV | ultraviolet |
| v/v | volume/volume |
| w/v | weight/volume |
| w/w | weight/weight |
| YPD | Yeast Extract-Peptone-Dextrose |
| α | significance level |
| β | beta |

# 1.0 CHAPTER 1

# INTRODUCTION

One of the most common monoterpene alcohol or terpineol found in nature is linalool. Linalool (IUPAC: 3,7-dimethyllocta-1,6-dien-3-ol) is made up of two isoprene units which have a molecular formula of $C_{10}H_{16}$ (Pubchem, 2013). Linalool is an isomer of geraniol and nerol. The only thing that differentiates linalool from geraniol and nerol is the location of the hydroxyl functional group (Agarwal, 2010). Linalool has a hydroxyl functional group on the third carbon, which makes linalool a tertiary alcohol (Peace Rhind, 2012). Due to its scent and flavouring properties, linalool was commonly added to processed food and beverage as flavouring, fragrance agent in cosmetics, perfumes and shampoo as well as household detergents. Besides, linalool often used to aid sleep and relaxation in aromatherapy as well as the synthesis of vitamin E in the body (Colbert, n.d.).

Linalool is normally obtained by undergoing fractional distillation and consequent rectification from oils of Cajenne rosewood, Brazil rosewood, Mexican linaloe, *Cinnamomum camphora* and coriander seeds (Burdock & Fenaroli, 2009). It can also be produced synthetically from geraniol and nerol by ortho-vanadate-catalysed isomerization (Kroschwitz, 2007). However, fractional distillation is relatively expensive due to the maintenance of the fractionating column. The process involved high temperature and high pressure which makes this method extremely dangerous (Kinyanjui, n.d.). A recent study was done on the selection and optimization of engineered-yeast strains for the production of foreign monoterpene, linalool (Rico, Pardo & Orejas, 2010). It showed that there was a gradual increase of recombinant linalool production, which made *Saccharomyces cerevisiae* a suitable host to synthesize various monoterpenes by expressing the suitable plant monoterpene synthases (MTS) in exchange for linalool synthase. This involved DNA manipulations, construction of plasmids and analysis of monoterpenes (Rico, Pardo & Orejas, 2010).

Nowadays, most industrial-scale fermentation uses feed stocks containing high sugar concentration as the growth medium. This would reduce the production cost compared to the use of basal medium such as nutrient agar or nutrient broth. Among all the yeast species, *S. cerevisiae* is the most often used yeast species in fermentation process due to its rapid growth rate, high fermentation rate and tolerate high concentration of ethanol as well as low pH (Matsushika, Goshima & Hoshino, 2014). According to Malaysian Pineapple Industry Board (MPIB), Malaysia is one of the world pineapple suppliers. It is reported that in Malaysia, there are approximately 96,957 metric tonnes of fresh pineapple production and 17,165 metric tonnes of canned pineapple produced in 2011 (Freshplaza, 2013). Linalool can be extracted from the fermentation of pineapple variety Josapine (Thiri, 2013; Ruhil, 2014). Josapine was introduced by Malaysian Agriculture Research and Development Institute (MARDI) in 1996 (Mohammed Selamat, 1996). A fresh ripe pineapple fruit has high content of vitamin C, total sugar, starch, ascorbic acid as well as volatile aroma compound that responsible for the fragrance of pineapple. Hence, this makes Josapine an ideal medium to be used in this study for linalool production. Studies were done on the linalool production of fermentation of Josapine juice and peel. However, no study was carried out on the β-glucosidase activity of fermented pineapple.

The aims of this study were:

- to carry out microbial and natural fermentation on pulp and peel of Josapine,
- to determine and compare the activity of beta (β)-glucosidase in both microbial and natural fermentation using p-nitrophenyl-β-D-glucopyranoside (pNPG) as substrate,
- to quantify the amount of β-glucosidase using spectrophotometric method and relate it to the amount of linalool present in pulp and peel of Josapine, and
- to quantify the amount of linalool extracted from pulp and peel of Josapine by spectrophotometric method.

# 2.0 CHAPTER 2

# LITERATURE REVIEW

## 2.1 TERPENES

Terpenes, also referred to as terpenoids, are the biggest group of natural compounds. They are mainly present in plants as ingredients of essential oils. Most terpenes are hydrocarbons while some are oxygen-containing compounds such as alcohols, aldehydes or ketones (Kriste, 2003). According to literature, there are approximately 30,000 terpenes known at present (Breitmaier, 2006). All terpenes are synthesized from isoprene, a five-carbon ($C_5$) building blocks which build up the carbon skeleton of terpenes (Zhang & Demain, 2005).

There are different types of terpenes which are differentiated by the number of isoprene units present. Those terpenes are hemi- ($C_5$), mono- ($C_{10}$), sesqui- ($C_{15}$), di- ($C_{20}$), sester- ($C_{25}$), tri- ($C_{30}$), tetra- ($C_{40}$) and polyterpenes $(C_5)_n$ where $n > 8$ (Breitmaier, 2006). Monoterpenes, sesquiterpenes, diterpenes, triterpenes and tetraterpenes are more common than other terpenes. Monoterpenes such as limonene and linalool are commonly used as fragrance agents in perfumes, antibacterial agents and essential oil (Zhuang, 2013). Sesquiterpenes such as farnesol mostly used in pharmaceutical as antibacterial and antifungal agent (Zhuang, 2013). Gibberellins are diterpenes which commercially used as feedstock for chemical applications in industries (Zhuang, 2013). Triterpenes include sterols, botryococcene, hopanoids and squalene used as biofuel, biologic markers and skin moisturizers in cosmetics (Zhuang, 2013). Tetraterpenes such as lycopene and beta-carotene can be found in food additives and food coloring (Zhuang, 2013).

There are two pathways in which terpenes can be synthesized. The two pathways are mevalonic acid (MVA) and 2-c-methyl-D-erythritol-4-phosphate (MEP) pathways (Dewick, 2002). The biosynthesis of terpenes takes place from two $C_5$ precursors which are isopentyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (Dewick, 2002).