UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF RHAMNOSE FROM POMELO PEEL USING LOCALLY-ISOLATED FUNGI Trametes sp. IP3

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FBSB 2016 47

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By

NUR HAZIQAH ANIYAH BINTI SALIHAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for Master of Science

February 2016

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DEDICATION

This work is dedicated to my beloved family, who has always given me courage and support to carry out my studies. Thanks to their everlasting love and care.

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfilment of the requirement for degree of Master of Science

PRODUCTION OF RHAMNOSE FROM POMELO PEEL USING LOCALLY-ISOLATED FUNGI *Trametes* sp. IP3

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February 2016

Chairman : Helmi Wasoh @ Mohamad Isa, PhD
Faculty : Biotechnology and Biomolecular Sciences

Pomelo fruit (*limau bali*) is very popular due to its huge flesh compared to the typical citrus fruit, orange. Malaysia produce in average around 8 000 tons of pomelo each year. However, because pomelo has a very thick skin, it was not highly demand by the industry. Despite its think skin (peel), the peel contains a rich source of flavonoid; naringin. Naringin, a bitter taste of flavonoid can be biodegraded into rhamnose and glucose with the presence of naringinase enzyme; β-glucosidase and α-L-rhamnosidase. Rhamnose is a typical 6-deoxyhexose similar to fucose and exhibits a taste similar to D-mannose and usually found in plant as a constituent of glycosides such as naringin. Many researches use chemical hydrolysis to obtain rhamnose from naringin. Rhamnose can be used to produce food flavour such as 4-hydroxy-2,5-dimethyl-furanone (HDMF) that was used abundantly in food flavor production for example Nestle as caramel flavour. Due to the abundance of acid used for the production of the food flavor, the process is not suitable for environmental friendly food production. As an alternative, rhamnose was obtained through microbial hydrolysis using enzyme from fungi. Therefore, this study was done to produce rhamnose and glucose using microbial (isolated fungi) process using the pomelo waste product (peel) as the main substrate with the minimal usage of harzardous chemical. In this study, 84 strains were isolated and it was found that strain IP 3 was able to produce 18.15 g/L rhamnose from 100 g/L dry treated pomelo peel, and 8.96 g/L of rhamnose from untreated pomelo peel. *Trametes* sp. IP3 has the potential to produce high naringinase enzyme for the bioconversion of naringin into rhamnose. However, the production of glucose was not reported with the production of rhamnose. Thus, a hydrolysate containing high activity (53.4 U/mL) of β-glucosidase enzyme from fermentation of *Ganoderma lucidum* (compost OPEFB as the substrate) was added to the fermentation media at day 6. Additional sugars were detected at the end of experiment; xylose, mannose, and rhamnose however, glucose was not detected. Nevertheless, *Trametes* sp. IP3 possesses the ability to produce sugars besides rhamnose with the presence of high activity of β-glucosidase enzyme.

Keywords: Rhamnose, naringin, pomelo, naringinase, α-L-rhamnosidase

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

PENGHASILAN RHAMNOSE MENGGUNAKAN KULIT LIMAU BALI YANG DIREMBESKAN OLEH KULAT *Trametes* sp. IP3

Oleh

NUR HAZIQAH ANIYAH BINTI SALIHAN

Februari 2016

Pengerusi : Helmi Wasoh @ Mohamad Isa, PhD
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Pomelo (limau bali) sangat popular disebabkan oleh saiz isinya yang lebih besar berbanding buah-buahan sitrus biasa seperti buah oren. Malaysia menghasilkan kira-kira 8 000 tan limau bali setiap tahun. Walau bagaimanapun, limau bali tidak menjadi tarikan dan pilihan industri makanan untuk tujuan pengkomersilan disebabkan oleh kulitnya yang sangat tebal. Walaubagaimanapun, kulit limau bali mengandungi sumber yang kaya dengan flavonoid iaitu naringin. Naringin, flavonoid yang agak pahit boleh di uraikan kepada rhamnose dan glukosa dengan kehadiran enzim naringinase; ß-glucosidase dan α-L-rhamnosidase. Rhamnose adalah sejenis 6-deoxyhexose seperti fucose dan mempunyai rasa yang sama seperti D-mannose. Rhamnose biasanya ditemui dalam tumbuhan sebagai satu daripada komponen glikosida seperti naringin. Banyak kajian dijalankan menggunakan hidrolisis kimia untuk mendapatkan rhamnose daripada naringin. Rhamnose boleh digunakan untuk menghasilkan rasa makanan seperti 4-hydroxy-2,5-dimetil-furanone (HDMF) dan banyak digunakan industri makanan seperti Nestle sebagai rasa karamel. Oleh kerana banyak asid yang digunakan dalam proses pembuatan perasa makanan tersebut, proses itu tidak sesuai untuk pengeluaran makanan mesra alam sekitar. Sebagai alternatif, rhamnose diperolehi melalui isolasi mikrob daripada limau bali untuk menghasilkan enzim ß-glucosidase dan α-L-rhamnosidase. Oleh itu, kajian ini dilakukan untuk menghasilkan dan glukosa rhamnose dengan bantuan mikrob (kulat) dengan menggunakan limau bali (kulit) sebagai substrat utama dengan penggunaan bahan kimia secara minimum. Dalam kajian ini, daripada 84 kulat, kulat jenis IP 3 dapat menghasilkan 18.15 g/L rhamnose dari 100 g/L kulit limau bali yang di rawat, dan 8.96 g/L rhamnose dari kulit limau bali tidak dirawat. *Trametes* sp. IP3 dikenalpasti mempunyai potensi untuk menghasilkan enzim naringinase tinggi untuk menukarkan naringin kepada rhamnose. Walau bagaimanapun, pengeluaran glukosa tidak dilaporkan dengan pengeluaran rhamnose. Oleh itu, hidrolisat yang mengandungi aktiviti yang tinggi (53.4 U/mL) daripada β-glucosidase enzim dari penapaian *Ganoderma lucidum* (kompos OPEFB sebagai substrat) telah ditambah kepada media penapaian pada hari ke enam. Gula tambahan

telah dikesan pada akhir eksperimen; xylosa, mannosa dan rhamnosa bagaimanapun, glukosa tidak dapat dikesan. Walau bagaimanapun, *Trametes* sp. IP3 mempunyai keupayaan untuk menghasilkan gula selain rhamnose dengan kehadiran aktiviti yang tinggi β-glucosidase enzim.

Kata kunci: Rhamnose, naringin, limau bali, naringinase, α-L-rhamnosidase

ACKNOWLEDGEMENT

Assalamualaikum wt wb.,

Thank you Allah for giving me strength and patience to finish my Master study. I would like to acknowledge my supportive supervisor, Dr. Helmi Wasoh @ Mohamad Isa for the endless guidance and care throughout my master studies. Thank you for showing the right way of doing research especially polishing my writing skills.

I would also like to acknowledge my co-supervisor Dr. Mohd Rafein Zakaria and Prof. Dr. Suraini Abd-Aziz for their guidance. Million thanks to our caring lab assistance Mr. Rosli Aslim, Mrs. Aluyah Marzuki, Mrs. Renuga Panjamurji, Mrs. Norazlina Mohamad and Mrs. Noraishah Abd Rahman for their kind assistance and suggestion throughout my study.

I would also like to acknowledge Ministry of Higher Education for funding this project through Fundamental Research Grant Scheme (FRGS).

Thank you to my dearest friends; Nordiyana Nordin, Faiqah Abd Rahim and all my friends, whom are very supportive, understanding, and comment. A special thanks to my parents and my husband, Mohd Rafiddy bin Miswan for their endless support and encouragement.

Thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment for 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

g	Gram
L	Litre
Min	Minutes
mL	Milliliter
nm	Nanometer
^{o}C	Degree Celsius
Rpm	Rotation per minute
OPEFB	Oil palm empty fruit bunch
g/L	Gram per liter
HPLC	High performance liquid chromatography
B	Beta
α	Alpha
U/mL	Unit per milliliter
SEM	Scanning electron microscope
dTDP	deoxythymidine diphosphate
DME	Dry methalonic extraction
UDP	Uridine diphosphate glucose
µm	Micrometer
EC	Enzyme commission number
PD	Potato dextrose
DNA	Deoxyribonucleic acid

CHAPTER 1

INTRODUCTION

1.1 Pomelo and Alternative Source of Rare Sugar (Rhamnose)

Pomelo (*Citrus maxima*) is a native citrus fruit to southern East Asia. In Malaysia, this Rutaceae family plant is widely grown in Perak, Kedah, Melaka and Johor. Pomelo peel consists of two major parts; flavedo and albedo. Flavedo, a green layer located at the outer side of fruit and albedo is a white layer with a spongy peel texture located between juice sacs and a flavedo (Panmanas and Charoonpong, 2012).

Every year massive peel is disposed as waste due to high consumption of pomelo flesh and causes a severe environmental issues (Hameed *et al.,* 2008). However, Dong-Mei *et al.,* (2011) stated that naringin, the main component in pomelo peel albedo can be utilized to produce rhamnose (rare sugar) and glucose by using naringinase enzyme (α-l-rhamnosidase and β-d-glucosidase). Therefore, this has opened an opportunity for the agriculture waste industry to maximize the application of waste and use it to produce other useful products. L-rhamnose (6-deoxy-mannose) is a rare sugar that had been used as a starting material in synthesis of organic compound. It is considered as a high potential substrate in producing flavor. A highly quality flavor, furaneol (trademark of Firmenich SA, Geneva) was using rhamnose as its precursor. It acts as a chiral building block in chemical synthesis, the deoxysaccharide that can only be obtained with great effort by chemical means. It is produced industrially from a variety of vegetable raw materials, for example from rutin or from hesperidin and naringin (Lang and Wullbrandt, 1999). In chemical process, production of rhamnose involves large quantities of potentially toxic and dangerous waste products. The process of waste management cost the industry sometimes more than the production cost since waste need to be treated before it can be properly disposed.

Rhamnose had been produced by several bacteria from rhamnolipid. However, rhamnose that produced by pathogenic bacteria is not favourable in food industries (Giraud and Naismith, 2000) since it might cause harm to the consumers. Therefore, using the abundance of inexpensive substrate (albedo from pomelo peel) as the main carbon source is potentially used to produce high amount of rhamnose. In this study, the effects of different extraction process and the production of rhamnose using pomelo peel were also studied.

Naringinase however is one of the expensive enzyme and by culturing excellent strains, high yield naringinase can be produced and decrease production cost. Some researchers had suggested strain *Aspergillus niger* (Bram and Solomons, 1965), however Tselenis-Kotsowilis *et al.* (1982) reported that there have been some

defects which restricted the application of the naringinase involving immobilized cost, poor stability, and equipment used. Therefore, new strains which are safe, easy to maintain and convenient for fermentation process have been isolated.

As stated before, naringin can be further hydrolyzed to glucose by hydrolysis of β-d-glucosidase (Vila Real *et al.*, 2007). However, Hakasson *et al.* (2011) stated that the presence of α-L-rhamnosidase will only inhibit the activity of β-glucosidase enzyme. Therefore, in this study, high β-glucosidase enzyme was extracted from other fermentation and was added into the rhamnose producing media to study whether the enzyme might help in producing glucose from naringin from hydrolysate.

It is generally known that cellulose can be converted into glucose by cellulase such as β-glucosidase. Cellulase has a great potential in industrial sector which can be used in textile, paper and poultry industry. The enzymatic breakdown of substrates by β-glucosidase brings the most promising technology for the conversion of lignocellulosic waste biomass (Baharuddin *et al.*, 2009a). In this work, further degradation of pomelo peel was carried out using cellulase from *Ganoderma lucidum*, a well-known fungi having the ability to grow well on oil plam tree. The hydrolysate of the final product was monitored using HPLC in order to check the quality of rhamnose. It is hope that this work will provide useful knowledge on the usage of pomelo peel as potential substrate for rhamnose production in the future.

The objectives of this study are:

a) To isolate and identify rhamnose producing fungi from rotten pomelo peel.

b) To produce rhamnose using albedo from pomelo peel through liquid state fermentation.

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