PRODUCTION OF FRUCTOOLIGOSACCHARIDES (FOS) BY ENZYMATIC REACTION FROM PHYTOENZYME OF *ANANAS COMOSUS* WASTE

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We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science in Bioprocess.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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Thesis submitted in fulfillment of the requirements
for the award of the degree of
Master of Science

Faculty of Chemical and Process Engineering Technology

UNIVERSITI MALAYSIA PAHANG

DECEMBER 2019

ACKNOWLEDGEMENTS

In the Name of Allah, the Beneficent, the Merciful.

First praise is to Allah, the Almighty, on whom ultimately we depend for sustenance and guidance. Second, my sincere appreciation goes to my supervisor Dr.Noormazlinah Binti Ahmad, whose guidance, careful reading and constructive comments was valuable. Her timely and efficient contribution helped me shape this into its final form and I express my sincerest appreciation for her assistance in any way that I may have asked.

I also wish to thank the Faculty of Chemical and Natural Process Engineering Technology, its leadership and the staff for providing me with an academic base, which has enabled me to take up this study. I am particularly grateful to technical staffs in the laboratory for their worthy contribution. I am also indebted to my colleagues at who helped me in many ways and made my stay in UMP pleasant and unforgettable.

Special thanks, tribute and appreciation to all those their names do not appear here who have contributed to the successful completion of this study. Finally, I'm forever indebted to my parents Haji Mat Jusoh Bin Abdul Ghani and Hajjah Che Kamariah Bt Abdullah and also to my siblings who, understanding the importance of this work suffered my hectic working hours. They were always supporting me and encouraging me with their best wishes.

I am also indebted to the Kementerian Pengajian Tinggi (KPT) and Universiti Malaysia Pahang (UMP) for funding my study and Postgraduate Grant Research Scheme (PGRS

ABSTRAK

Fructooligosaccharides (FOS), yang dianggap sebagai fruktans jenis inulin, merupakan sumber penting bagi sebatian prebiotik, yang digunakan secara meluas sebagai bahan dalam makanan berfungsi. Prebiotik dianggap sebagai makanan berfungsi tambahan kerana kemampuan untuk menggalakkan kesihatan perumah dengan meningkatkan pertumbuhan mikroorganisma probiotik. Dalam kajian ini, FOS dihasilkan dengan menggunakan tindak balas enzim dan fitoenzim dari sisa *A. Comosus*. Pencirian invertase dan fitoenzim dari sisa *A.Comosus* telah dilaksanakan sebelum pengeluaran FOS dengan menggunakan FTIR, HPLC, UV-Vis, SDS-PAGE dan memperoleh FOS daripada fitoenzim dan invertase mengandungi kompleks sebatian protein. Keputusan FTIR menunjukkan bahawa kedua-dua invertase dan fitoenzim dari sisa *A.comosus* membentang dari 1640.60 cm^{-1} dan 1649.99cm^{-1} masing-masing dan ia adalah ikatan N-H. Profil kromatografi produk reaksi dari fitoenzim sisa *A.comosus* menunjukkan kehadiran sukrosa, fruktosa, glukosa dan FOS iaitu kestose, dan nystose.Penghasilan FOS diteruskan oleh kaedah statistik OFAT, FFD dan statistik dengan parameter pengoptimalan yang merupakan, kepekatan sukrosa, pH, kepekatan fitoenzim dan suhu dengan menggunakan Design Expert versi 7.0. Kajian OFAT menunjukkan bahawa masa tindak balas, kepekatan substrat, kepekatan fitoenzim, suhu, pH adalah tindak balas yang ketara untuk menghasilkan FOS dengan nilai 100 minit, 60% (w/v), 30% (w/v) ,75˚C, dan pH 6.0. Kesemua tindak balas ini dikaji lagi oleh kaedah FFD yang menunjukkan bahawa kepekatan fitoenzim, pH, kepekatan sukrosa, kepekatan fitoenzim dan suhu adalah tindak balas yang signifikan untuk menghasilkan FOS. Kaedah CCD mendapati bahawa pH (6) dan suhu 60˚C menghasilkan maksimum FOS pada 211.334 (g/ml). Pengesahan persamaan empirikal yang diperoleh daripada kajian pengoptimuman dilakukan dalam skala makmal dengan hanya ralat 0.828%. Sebagai kesimpulan, kajian ini berjaya menghasilkan FOS menggunakan pendekatan baru dan sumber alternatif yang berasal dari fitoenzim dari sisa *A.Comosus*

ABSTRACT

Fructooligosaccharides (FOS), which are considered as inulin-type fructans, represent an important source of prebiotic compounds that are widely used as an ingredient in functional foods. Prebiotics are considered as additives of functional food due to the ability to promote host health by increasing the growth of probiotic microorganisms. In this study, FOS were produced by using enzymatic reaction of the phytoenzymes from *Ananas Comosus* waste.The characterisation of invertase and the phytoenzymes of *A. Comosus* waste was performed prior to FOS production by using Fourier transfrom infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC), and ultraviolet-visible (UV-Vis) spectroscopy. FOS obtained from phytoenzymes and invertase showed similarities in terms of complex protein compounds. FTIR results showed that both invertase and the phytoenzymes from *A.comosus* waste give stretching from 1640.60 cm^{-1} and 1649.99cm^{-1} respectively and it is N-H bond. The chromatographic profile of the reaction product from the phytoenzyme of *A.comosus* waste revealed the presence of sucrose, fructose, glucose and FOS namely, kestose, and nystose. The production of FOS proceeds by fractional factorial design (FFD) central composite design (CCD) statistical methods with the optimisation parameters of reaction time, sucrose concentration, pH, phytoenzyme concentration and temperature using Design Expert version 7.0. All of these responses were screened by FFD method which showed that all the parameters were significant response to produce FOS. CCD method evaluates that pH (6) and temperature 60˚C was producing maximum FOS at 211.334(g/mL). The validation of empirical equations gained from the optimisation study was performed in laboratory scale with only 0.828 % error. As a conclusion, this study has successfully produced FOS using a new approach of alternative source which is from the phytoenzymes of *A.Comosus* waste.

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LIST OF ABBREVIATIONS

A.comosus	A.comosus
ANOVA	Analysis of variance
BOD	Biological Oxygen Demand
CCD	Central Composite Demand
COD	Chemical Oxygen Demand
DNS	Dinitrosalicylic acid
FFD	Fractional Factorial Design
FOS	Fructooligosaccharides
Ftase	Fructosyltransferase
FTIR	Fourier Transform Infrared
GF_2	Kestose
GF_3	Nystose
GF_4	1^F-Fructosyltransferase
HPLC	High Performance Liquid Chromatography
K_M	Michaelis constant
LPNM	Lembaga Perindustrian Nenas Malaysia
OD	Optical Density
OFAT	One Factor One Time
RSM	Response Surface Methodology
SDS-PAGE	Sodium dodecyl sulphate –polyacrylamide gel electrophoresis
UV-Vis	Ultra Violet Visible Spectroscopy
V_{max}	Maximum rate of reaction

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