OPTIMISATION OF ENZYMATIC REACTIONS FOR GALACTO-OLIGOSACCHARIDES SYNTHESIS USING BETA-GALACTANASE FROM *Geobacillus mahadii* Geo-05

NOOR FAIZAH ISMAIL

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Bioprocess Engineering)

School of Chemical and Energy Engineering
Faculty of Engineering
Universiti Teknologi Malaysia

APRIL 2022

DEDICATION

Buat yang tersayang…

♥*Ayahanda Ismail Dol & Bonda Zaiton Hassan*

♥*Noor Atyqah, Husnul Hadey, Nurulain, Husnul Hafiz, Nurul Izzaty*

ACKNOWLEDGEMENT

All praises belong to Allah, The Almighty and The Benevolent♥.

Life is a journey that must be travelled no matter how hard it seems. It has been a long way, and I am grateful to finish the most incredible journey that I have started. “Take the good with the bad, as only with darkness we can see shining stars”.

In particular, to my PhD supervisor, Assoc. Prof. Dr. Dayang Norulfairuz Abang Zaidel, my gratitude goes far beyond words for your guidance, patience, motivation, and acceptance me as your student. My appreciation also goes to Prof. Dr. Rosli Md Illias, for the valuable comments and allowing me to be part of Labatasbukit’s members. Not to forget, Prof. Dr. Muhammad Mukram Mohamed Mackeen and his team in UKM helped me in the early stage of my experiment. My special thanks to Fisheries Research Institute senior officers for their courage, advice and understanding while balancing my responsibilities as a researcher and being a student.

I am also grateful to the former Ministry of Higher Education Malaysia for the MyBrain15 scholarship and UTM for giving me the opportunity to conduct my PhD study here. Lab technicians and staff also deserve special thanks for their assistance in completing my study. Last but not least, no words can describe the unconditional love of my family, especially my father and mother, who never give up on me. Thank you for your endless praying and support. To my friends, my besties, you know who you are; wherever we are, our memories remain. Having someone who is always by your side is precious. I am blessed to have them until the end.

ABSTRACT

Galacto-oligosaccharide (GOS) is one of the prominent prebiotics used in infant formula milk. Endo-beta-1,4-galactanase hydrolyses galactan and produces GOS with different degree of polymerisation (DP). DP of the GOS could vary depending on the enzyme source. Besides, reaction parameters such as enzyme loading, substrate loading, incubation temperature, pH and reaction time also contribute to various DP of GOS synthesis. In this study, beta-galactanase from *Geobacillus mahadii* Geo-05 was synthesised with a codon optimised sequence (named BgcGC) and biochemically characterised for its optimum function. The BgcGC reaction was optimised to obtain the highest yield of the targeted GOS (galactotriose, G3 and galactotetraose, G4) from the potato galactan. Then, the optimised conditions were applied on pectin extracted from sweet potato peels (SPP) to produce the GOS. BgcGC could actively hydrolyse potato galactan at 60°C in McIlvaine buffer pH 6 and stable to a wide range of pH between 4 to 10. According to the screening by the factorial design, three reaction parameters significantly contributed to the highest yield of GOS. Due to curvature prediction, the central composite design was further applied to optimise BgcGC reaction to produce GOS. The optimum reaction parameters for the GOS synthesis were identified at 0.15 U/ml BgcGC loading, 1.5 h incubation time and 50°C reaction temperature yielding a 1.5-fold increment of the GOS synthesis. The reaction of BgcGC towards SPP pectin resulted in a 0.081 mg/g yield of G3. These findings showed that GOS synthesised by BgcGC could be enhanced through the manipulation of reaction parameters with a notable increment of GOS yield. Moreover, BgcGC exhibited the potential to be used as an enzyme cocktail for the depolymerisation of pectin polysaccharides.

ABSTRAK

Galakto-oligosakarida (GOS) adalah salah satu prebiotik penting yang digunakan dalam susu formula bayi. Endo-beta-1,4-galaktanase menghidrolisis galaktan dan menghasilkan GOS dengan darjah pempolimeran (DP) yang berbeza. Perbezaan DP GOS bergantung kepada sumber enzim. Selain itu, parameter tindak balas seperti muatan enzim, muatan substrat, suhu inkubasi, pH dan tempoh tindak balas juga menyumbang kepada sintesis GOS dengan pelbagai DP. Dalam kajian ini, beta-galaktanase daripada *Geobacillus mahadii* Geo-05 telah disintesis dengan jujukan kodon-optimum (dinamakan sebagai BgcGC) dan dicirikan secara biokimia untuk fungsinya yang optima. Tindak balas BgcGC dioptimumkan untuk memperoleh hasil GOS tertinggi (galaktotriose, G3 dan galaktotetraose, G4) daripada galaktan kentang. Kemudian, keadaan optima telah digunakan ke atas ekstrak pektin dari kulit ubi keledek (SPP) untuk menghasilkan GOS. BgcGC aktif menghidrolisis galaktan kentang pada suhu 60°C dalam larutan McIlvaine pH 6 dan mempunyai julat kestabilan pH yang besar di antara 4 hingga 10. Berdasarkan saringan reka bentuk faktorial, terdapat tiga parameter tindak balas yang penting dalam menyumbang kepada hasil GOS yang tertinggi. Disebabkan jangkaan kelengkungan, reka bentuk komposit berpusat seterusnya digunakan untuk mengoptimumkan tindak balas BgcGC bagi menghasilkan GOS. Parameter tindak balas optima untuk sintesis GOS telah dikenalpasti pada muatan 0.15 U/ml BgcGC, tempoh tindak balas 1.5 jam dan suhu inkubasi 50°C menghasilkan peningkatan 1.5 kali ganda sintesis GOS. Tindak balas BgcGC terhadap pektin SPP menghasilkan 0.081 mg/g G3. Penemuan ini menunjukkan sintesis GOS oleh BgcGC boleh ditingkatkan menerusi manipulasi parameter tindak balas dengan peningkatan hasil GOS yang ketara. Tambahan pula, BgcGC mempamerkan potensi untuk digunakan sebagai koktail enzim bagi penyahpolimeran polisakarida pektin.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

2-ME	-	2-mercaptoethanol
ABEE	-	*p*-aminobenzoic acid ethyl ester
ANOVA	-	analysis of variances
AP	-	apple pomace
A_x	-	absorbance
BgcGC	-	beta-galactanase with codon-optimised from *Geobacillus mahadii* Geo-05
BLAST	-	basic local alignment search tool
bp	-	base pair
BSA	-	bovine serum albumin
CAI	-	codon adaptation index
Ca^{2+}	-	calcium ion
CCD	-	central composite design
Co^{2+}	-	cobalt ion
Cu^{2+}	-	cuprum ion
Da	-	dalton
DA	-	debranched arabinan
DNA	-	deoxyribonucleic acid
DOE	-	design of experiment
DP	-	degree of polymerisation
EC	-	enzyme commission
EDTA	-	ethylenediaminetetraacetic acid
FD	-	factorial design
Fe^{2+}	-	ferrous ion
g	-	gram
GH	-	glycoside hydrolase
G1	-	galactose
G2	-	galactobiose
G3	-	galactotriose
G4	-	galactotetraose
G5	-	galactopentaose

GOS	-	galacto-oligosaccharide
h	-	hours
HPLC-UV	-	high-performance liquid chromatography-ultra violet
H_2SO_4	-	sulfuric acid
IMAC	-	immobilised metal affinity chromatography
IPTG	-	isopropyl β-D-1-thiogalactopyranoside
k_m	-	Michaelis-Menten constant
k_{cat}/k_m	-	catalytic efficiency
K^+	-	potassium ion
LB	-	luria bertani
LG	-	lupin galactan
M	-	molar
Mg^{2+}	-	magnesium ion
min	-	minutes
Mn^{2+}	-	manganese ion
MW	-	molecular weight
NEB	-	new england biolabs
Ni-NTA	-	nickel-nitriloacetic acid
Ni^{2+}	-	nickel ion
n.s	-	not specified
OD	-	optical density
OFAT	-	one-factor-at-a-time
PAHBAH	-	*p*-hydroxybenzoic acid hydrazide
PA	-	pectin from apples
PG	-	potato galactan
pH	-	potential of hydrogen
pI	-	isoelectric point
PMSF	-	phenylmethylsulfonyl fluoride
ppm	-	parts per million
psi	-	pounds per square inch
RGI	-	rhamnogalacturonan I
RID	-	refractive index detector
rpm	-	revolutions per minutes

RSM	-	response surface methodology
SDS-PAGE	-	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sp.	-	species
SPP	-	sweet potato peels
TFA	-	trifluoro-acetic acid
v	-	volume
V_{max}	-	maximum velocity
vs.	-	versus
w	-	weight
Zn^{2+}	-	zinc ion
µg	-	microgram
µl	-	microliter
µm	-	micrometre
%	-	percentage
°C	-	degree celsius

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Pectin, a complex polysaccharide found in the plant cell walls, is categorised into four main regions: homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II and xylogalacturonan (Mohnen, 2008; Reichembach and de Oliveira Petkowicz, 2021). Depolymerisation of pectin structure requires different types of enzymes such as arabinofuranosidase, endo-polygalacturonase and beta-galactanase to work on the specific group of substrate. Galactan exists as side chains attached to the rhamnogalacturonan I domain with galactose as a sugar monomer. Beta-galactanase is responsible for the hydrolysis of beta-linkage in galactan into galactose and galacto-oligosaccharide (GOS). GOS mimics the function of oligosaccharides found in human milk and has been extensively studied using a by-product from cheese and casein industry, i.e. lactose as substrate. It is derived from the transgalactosylation reaction of beta-galactosidase and enhanced growth of beneficial bacteria in the human gut (Carević *et al*., 2018; Fara *et al*., 2020). GOS is also attained from galactan by the reaction of beta-galactanase, either by the hydrolysis or transglycosylation reaction.

Although chemical synthesis can be used to produce GOS, the enzymatic approach is always preferable by the industry such as FrieslandCampina and Bimuno manufacturers due to its specific target, mild condition and safe to be consumed. Nevertheless, the limitations of enzyme application at the industrial level are the capacity to operate at high temperatures, rough flow regimes and low product yield. One of the strategies to cater to the industrial need for the robust enzyme in terms of temperature stability is to isolate the enzyme from a thermophile environment, which gives a better chance to obtain a thermostable enzyme. The thermostable enzyme is essential for an enzymatic reaction to perform at high temperature, especially when

using a concentrated substrate that requires heat to solubilise, for example, high-methoxyl pectin (Raj *et al*., 2012). Besides, the reaction that runs at a high temperature could improve the solubility of the concentrated substrate and reduce the risk of microbial contamination (Kambourova, 2018; Liu *et al*., 2019).

Meanwhile, various degree of polymerisation (DP) of GOS has been reported and its diversity depends on many factors. DP refers to a number of monomers that builds up the oligosaccharide. Oligosaccharide with higher DP was postulated to arrive at the distal colon because it can resist acid hydrolysis during transportation (Martins *et al*., 2019). DP equal to three and more, particularly trisaccharides, were preferred to be consumed by bifidobacteria (Cardelle-Cobas *et al*., 2009; O'Connell Motherway *et al*., 2013). Several strategies can be employed to obtain the targeted GOS and subsequently enhance the production yield. One of the approaches is through enzyme modification or protein engineering. Muderspach *et al*. (2021) found that more galactopentaose (G5) than G4 has been synthesised by extending the substrate binding groove of beta-1,4-galactanase compared to the wild type enzyme. Besides, enzyme selection is also essential to target a specific DP where subsites variation between fungal and bacterial beta-galactanases determine the length of GOS produced (Ryttersgaard *et al*., 2004).

Apart from that, reaction parameters also contribute to the formation of different DP and yield of GOS (Martins *et al*., 2019). Hydrolysis of potato galactan by the recombinant beta-1,4-galactanase from *Geobacillus stearothermophilus* had discovered the synthesis of higher molecular weight of GOS during the earlier time of incubation before it started to degrade into shorter GOS (Tabachnikov and Shoham, 2013). In contrast, a longer reaction time was needed by the recombinant beta-1,4-galactanase from *Clostridium thermocellum* to obtain an optimum yield of DP 4 of pectic galactan-oligosaccharides compared to DP 2 (González-Ayón *et al*., 2019). However, other parameters such as temperature, pH and enzyme to substrate ratio were varied accordingly. Thus, optimising the enzymatic reaction may need to look into the interaction between factors involved in the response affecting GOS synthesis. The statistical design allows the investigation of the relationship between parameters associated with the reaction.

1.2 Problem Statement and Novelty

GOS from plant-based is lactose-free because it does not come from a dairy source. Hence, it serves as an alternative prebiotic for a lactose-intolerance individual. The GOS synthesis by the transgalactosylation of beta-galactosidase on lactose is extensively studied while optimisation of beta-galactanase for the GOS synthesis with a defined DP is scarcely evaluated. Product from the beta-galactanase reaction contains galactose and GOS with various DP. One of the strategies to optimise the production of targeted GOS is by manipulating the enzymatic reaction parameters. Although the hydrolysis of galactan resulted in the formation of GOS, it can be further hydrolysed into a monomer of galactose. Galactose (G1), galactobiose (G2), galactotriose (G3) and galactotetraose (G4) were among the major products reported from the hydrolysis reaction of beta-galactanase towards galactan or galactan-rich polysaccharides. In this study, the targeted product was G3 and G4 and referred as GOS. Several factors such as reaction time, enzyme loading, and temperature affect the hydrolysis reaction and final product formation. These factors are sometimes interrelated and may or may not significantly affect GOS yield. Therefore, controlling the reaction conditions and studying the effect of interaction between parameters may result in a higher synthesis of GOS.

Apart from that, different enzymes have different features that affect the reaction. It holds the key to product specificity and how the reaction is conducted. Studying the characteristic of the enzyme is vital to help better understanding and manipulation of the enzyme reaction. For instance, the thermostable enzyme allows the reaction to occur at higher temperatures and reduces microbial contamination. It is also important when dealing with a concentrated substrate such as high-methoxyl pectin that requires heat to solubilise. Thus, this is the first report on the optimisation of GOS synthesis by a thermostable beta-galactanase with codon-optimisation from *Geobacillus mahadii* (*G. mahadii*) Geo-05 (hereafter named as BgcGC) focusing on G3 and G4 using potato galactan. In addition, the potential of BgcGC reaction for GOS synthesis using locally extracted pectin galactan from sweet potato peels also was the first has been reported here.

1.3 Objectives

The objectives of this study were:

a) To determine the characteristics of a recombinant beta-galactanase isolated from thermophilic bacteria, *G. mahadii* Geo-05.

b) To optimise reaction parameters of BgcGC hydrolysis towards commercial potato galactan for the GOS synthesis.

c) To evaluate the performance of BgcGC using pectin extracted from sweet potato peels (SPP) for the GOS synthesis.

1.4 Scope of Research

This study focuses on the characterisation of locally isolated beta-galactanase from *G. mahadii* Geo-05 and the improvement of GOS synthesis through optimisation of reaction conditions. Following were the scopes of this study:

a) Synthesis of gene; optimisation of BgcGC codon according to *Escherichia coli* expression system as the host. Screening of inducer, isopropyl β-D-1-thiogalactopyranoside (IPTG) concentrations (0.05-1 mM) and post-induction temperature (15-25°C) for overexpression of BgcGC. Purification of BgcGC using affinity and ion-exchange chromatography. Biochemical characterisation of purified BgcGC (optimum temperature and stability, optimum pH and stability, effect of metal ions and additives, substrate specificity and kinetic analysis).

b) Preliminary experiment using one-factor-at-time (OFAT) to obtain a range of parameters involved in the reaction of BgcGC. They were enzyme loading (0.1-4.0 U/ml), substrate loading (0.2-2.0% w/v), temperature (20-80°C), pH (2-10), time of reaction (10 m - 6 h). Screening for significant parameters using fractional factorial design (FD). Optimisation of reaction processes of GOS

synthesis using response surface methodology (RSM) of three significant parameters, i.e. enzyme loading (0.05-0.15 U/ml), temperature (30-50°C) and time of reaction (1.5-4.0 h) to achieve an optimum yield of GOS (total of G3 and G4).

c) Extraction of pectin from sweet potato peels (SPP), *Ipomoea batatas* (L.) Lam using acid hydrolysis method (0.05 M HCl, 90°C, 1 h) and the reaction of SPP pectin using BgcGC to obtain GOS. Analysis of GOS using HPLC.

1.5 Significance of Study

Instead of lactose, galactan is also a substrate for the synthesis of GOS via the enzymatic reaction of beta-galactanase. The utilisation of plant biomass to produce value-added products is in line with sustainable development and waste minimisation. Beta-galactanase plays an important role in depolymerising pectin polysaccharide, specifically galactan presence as side-chains on rhamnogalacturonan I, part of the pectin component. Locally isolated beta-galactanase with thermostable property gives an advantage for application to be conducted at high temperature. In the meantime, optimisation of enzymatic reaction is the first step prior to up-scaling production. Thus, results from the present work support application of beta-galactanase on the hydrolysis of pectin galactan to produce higher GOS yield at optimum reaction conditions. The significant parameters contributed in enhancing GOS yield resulted from the hydrolysis reaction of BgcGC may become a referral for future work.

REFERENCES

Aachary, A. A., and Prapulla, S. G. (2009). Value addition to corncob: Production and characterization of xylooligosaccharides from alkali pretreated lignin-saccharide complex using *Aspergillus oryzae* MTCC 5154. *Bioresource Technology*, Elsevier Ltd, 100(2), 991–995.

Abang Zaidel, D. N., Hamidon, N. H., and Mat Zahir, N. (2017). Extraction and Characterization of Pectin from Sweet Potato (*Ipomoea batatas*) Peels using Alkaline Extraction Method. *Acta Horticulturae*, 1–8.

Abang Zaidel, D. N., Ismail, N. H., Mohd Jusoh, Y. M., Hashim, Z., and Wan Azelee, N. I. (2020). Optimization of sweet potato pectin extraction using hydrochloric acid. *Materials Science and Engineering*, 736, 1–10.

Abdul Manas, N. H., Md. Illias, R., and Mahadi, N. M. (2018). Strategy in manipulating transglycosylation activity of glycosyl hydrolase for oligosaccharide production. *Critical Reviews in Biotechnology*, Informa Healthcare USA, Inc, 38, 272–293.

Abou-Taleb, K. A., and Galal, G. F. (2018). A comparative study between one-factor-at-a-time and minimum runs resolution-IV methods for enhancing the production of polysaccharide by *Stenotrophomonas daejeonensis* and *Pseudomonas geniculate*. *Annals of Agricultural Sciences*, Elsevier B.V., 63, 173–180.

Akpinar, O., Erdogan, K., Bakir, U., and Yilmaz, L. (2010). Comparison of acid and enzymatic hydrolysis of tobacco stalk xylan for preparation of xylooligosaccharides. *LWT - Food Science and Technology*, Elsevier Ltd, 43, 119–125.

Al-Allaf, F. A., Tolmachov, O. E., Zambetti, L. P., Tchetchelnitski, V., and Mehmet, H. (2013). Remarkable stability of an instability-prone lentiviral vector plasmid in *Escherichia coli* Stbl3. *3 Biotech*, 3, 61–70.

Alam, M. K., Rana, Z. H., and Islam, S. N. (2016). Comparison of the Proximate Composition , Total Carotenoids and Total Polyphenol Content of Nine Orange-Fleshed Sweet Potato Varieties Grown in Bangladesh. *Foods*, 5(64), 1–10.

Alsina, C., Faijes, M., and Planas, A. (2019). Glycosynthase-type GH18 mutant chitinases at the assisting catalytic residue for polymerization of

chitooligosaccharides. *Carbohydrate Research*, Elsevier, 478, 1–9.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Gapped BLAST and PSI-BLAST: a new generation of protein database search programs*, 25(17), 3389–3402.

Altschul, S. F., Wootton, J. C., Gertz, E. M., Agarwala, R., Morgulis, A., Schäffer, A. A., and Yu, Y. K. (2005). Protein database searches using compositionally adjusted substitution matrices. *FEBS Journal*, 272, 5101–5109.

Amin, S. R., Erdin, S., Ward, R. M., Lua, R. C., and Lichtarge, O. (2013). Prediction and experimental validation of enzyme substrate specificity in protein structures. *PNAS*, E4195–E4202.

Andreani, E. S., Karboune, S., and Liu, L. (2021). Structural characterization of pectic polysaccharides in the cell wall of stevens variety cranberry using highly specific pectin-hydrolyzing enzymes. *Polymers*, 13(1842), 1–14.

Arcus, V. L., Prentice, E. J., Hobbs, J. K., Mulholland, A. J., Kamp, M. W. Van Der, Pudney, C. R., Parker, E. J., and Schipper, L. A. (2016). On the Temperature Dependence of Enzyme-Catalyzed Rates. *Biochemical Journal*, 55, 1681–1688.

Arnous, A., and Meyer, A. S. (2008). Comparison of methods for compositional characterization of grape (*Vitis vinifera* L.) and apple (*Malus domestica*) skins. *Food and Bioproducts Processing*, 86, 79–86.

Aydar, A. Y. (2018). *Chapter 10: Utilization of Response Surface Methodology in Optimization of Extraction of Plant Materials*. *INTECH*.

Ayechu-Muruzabal, V., van Stigt, A. H., Mank, M., Willemsen, L. E. M., Stahl, B., Garssen, J., and van't Land, B. (2018). Diversity of human milk oligosaccharides and effects on early life immune development. *Frontiers in Pediatrics*, 6(239), 1–9.

Balthazar, C. F., Silva, H. L. A., Vieira, A. H., Neto, R. P. C., Cappato, L. P., Coimbra, P. T., *et al*. (2017). Assessing the effects of different prebiotic dietary oligosaccharides in sheep milk ice cream. *Food Research International*, Elsevier Ltd, 91, 38–46.

Bandara, M. D., Stine, K. J., and Demchenko, A. V. (2019). The chemical synthesis of human milk oligosaccharides: Lacto-N-neotetraose (Galβ1→4GlcNAcβ1→3Galβ1→4Glc). *Carbohydrate Research*, Elsevier, 483, 107743.

Barbosa, F. C., Kendrick, E., Brenelli, L. B., Arruda, H. S., Pastore, G. M., Rabelo, S. C., Damasio, A., Franco, T. T., Leak, D., and Goldbeck, R. (2020). Optimization of cello-oligosaccharides production by enzymatic hydrolysis of hydrothermally pretreated sugarcane straw using cellulolytic and oxidative enzymes. *Biomass and Bioenergy*, 141(105697), 1–9.

Bartlett, G. J., Porter, C. T., Borkakoti, N., and Thornton, J. M. (2002). Analysis of catalytic residues in enzyme active sites. *Journal of Molecular Biology*, 324, 105–121.

Bartolozzi, A., and Seeberger, P. H. (2001). New approaches to the chemical synthesis of bioactive oligosaccharides. *Current Opinion in Structural Biology*, 11, 587–592.

Basso, A., and Serban, S. (2019). Industrial applications of immobilized enzymes — A review. *Molecular Catalysis*, Elsevier, 479(110607), 1–20.

Bateman, A., Martin, M. J., Orchard, S., Magrane, M., Agivetova, R., Ahmad, S., *et al*. (2021). UniProt: The universal protein knowledgebase in 2021. *Nucleic Acids Research*, Oxford University Press, 49, D480–D489.

Benkoulouche, M., Fauré, R., Remaud-Siméon, M., Moulis, C., and André, I. (2019). Harnessing glycoenzyme engineering for synthesis of bioactive oligosaccharides. *Interface Focus*, 9, 20180069.

Bernal, L. F., Lopez, G., Ruiz, M., Vera-Bravo, R., Reyes, A., and Baena, S. (2017). Response Surface Methodology (RSM) for analysing culture conditions of *Acidocella facilis* strain USBA-GBX-505 and Partial Purification and Biochemical Characterization of Lipase 505 LIP. *Universitas Scientiarum*, 22(1), 45–70.

Berrocal, C., Chico, H., Carranza, E., and Vega, R. (2021). Desirability function for optimization of the synthesis of high-panose isomaltooligosaccharides from maltose catalyzed by a novel commercial enzyme preparation from *Aspergillus niger*. *Biochemical Engineering Journal*, Elsevier B.V., 171(108003), 1–10.

Bhatwa, A., Wang, W., Hassan, Y. I., Abraham, N., Li, X. Z., and Zhou, T. (2021). Challenges Associated With the Formation of Recombinant Protein Inclusion Bodies in *Escherichia coli* and Strategies to Address Them for Industrial Applications. *Frontiers in Bioengineering and Biotechnology*, 9(630551), 1–18.

Bhuyan, A. K. (2009). On the Mechanism of SDS-Induced Protein Denaturation On the Mechanism of SDS-Induced Protein Denaturation. *Biopolymers*, 93(2), 186–

199.

Bissaro, B., Saurel, O., Arab-Jaziri, F., Saulnier, L., Milon, A., Tenkanen, M., Monsan, P., O'Donohue, M. J., and Fauré, R. (2014). Mutation of a pH-modulating residue in a GH51 α-L-arabinofuranosidase leads to a severe reduction of the secondary hydrolysis of transfuranosylation products. *Biochimica et Biophysica Acta - General Subjects*, Elsevier B.V., 1840, 626–636.

Bjerga, G. E. K., and Williamson, A. K. (2015). Cold shock induction of recombinant Arctic environmental genes. *BMC Biotechnology*, BMC Biotechnology, 15(78).

Bode, L., Contractor, N., Barile, D., Pohl, N., Prudden, A. R., Boons, G. J., Jin, Y. S., and Jennewein, S. (2016). Overcoming the limited availability of human milk oligosaccharides: Challenges and opportunities for research and application. *Nutrition Reviews*, 74(10), 635–644.

Böger, M., Hekelaar, J., Leeuwen, S. S. Van, Dijkhuizen, L., Lammerts, A., and Bueren, V. (2019). Structural and functional characterization of a family GH53 β-1,4-galactanase from *Bacteroides thetaiotaomicron* that facilitates degradation of prebiotic galactooligosaccharides. *Journal of Structural Biology*, Elsevier, 205(1), 1–10.

Bornhorst, J., and Falke, J. (2010). [16] Purification of Proteins Using Polyhistidine Affinity Tags. *Methods Enzymol.*, 326, 245–254.

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical biochemistry*, 72, 248–254.

Van Bueren, A. L., Mulder, M., Van Leeuwen, S., and Dijkhuizen, L. (2017). Prebiotic galactooligosaccharides activate mucin and pectic galactan utilization pathways in the human gut symbiont *Bacteroides thetaiotaomicron*. *Scientific Reports*, Nature Publishing Group, 7, 1–13.

Burgess-Brown, N. A., Sharma, S., Sobott, F., Loenarz, C., Oppermann, U., and Gileadi, O. (2008). Codon optimization can improve expression of human genes in *Escherichia coli*: A multi-gene study. *Protein Expression and Purification*, 59, 94–102.

Calsavara, L. P. V., Moraes, F. F., and Zanin, G. M. (2000). Thermal stability and energy of deactivation of free and immobilized cellobiase. *Brazilian Journal of Chemical Engineering*, 17, 4–7.

Cantarel, B. I., Coutinho, P. M., Rancurel, C., Bernard, T., Lombard, V., and Henrissat,

B. (2009). The Carbohydrate-Active EnZymes database (CAZy): An expert resource for glycogenomics. *Nucleic Acids Research*, 37, 233–238.

Cardelle-Cobas, A., Fernández, M., Salazar, N., Martínez-Villaluenga, C., Villamiel, M., Ruas-Madiedo, P., and De Los Reyes-Gavilán, C. G. (2009). Bifidogenic effect and stimulation of short chain fatty acid production in human faecal slurry cultures by oligosaccharides derived from lactose and lactulose. *Journal of Dairy Research*, 76(3), 317–325.

Cardelle-cobas, A., Olano, A., Irazoqui, G., Giacomini, C., and Corzo-martínez, M. (2016). Synthesis of Oligosaccharides Derived from Lactulose (OsLu) Using Soluble and Immobilized *Aspergillus oryzae* β-Galactosidase. *Frontiers in Bioengineering and Biotechnology*, 4(21), 1–10.

Carević, M., Vukašinović-Sekulić, M., Ćorović, M., Rogniaux, H., Ropartz, D., Veličković, D., and Bezbradica, D. (2018). Evaluation of β-galactosidase from *Lactobacillus acidophilus* as biocatalyst for galacto-oligosaccharides synthesis: Product structural characterization and enzyme immobilization. *Journal of Bioscience and Bioengineering*, 126(6), 697–704.

Carrillo, F., Lis, M. J., Colom, X., López-Mesas, M., and Valldeperas, J. (2005). Effect of alkali pretreatment on cellulase hydrolysis of wheat straw: Kinetic study. *Process Biochemistry*, 40, 3360–3364.

Cheng, D., Jiang, C., Xu, J., Liu, Z., and Mao, X. (2020). Characteristics and applications of alginate lyases: A review. *International Journal of Biological Macromolecules*, Elsevier B.V., 164, 1304–1320.

Chookaew, T., O-Thong, S., and Prasertsan, P. (2014). Statistical optimization of medium components affecting simultaneous fermentative hydrogen and ethanol production from crude glycerol by thermotolerant *Klebsiella* sp. TR17. *International Journal of Hydrogen Energy*, Elsevier Ltd, 39, 751–760.

Chung, C. ., Niemela, S. L., and Miller, R. H. (1989). One-step preparation of competent *Escherichia coli*: Transformation and storage of bacterial cells in the same solution. *Proceedings of the National Academy of Sciences of the United States of America*, 86, 2172–2175.

Chung, N. H., and Hoang, P. H. (2019). Preparation of oat spelt xylan and its application as additive for enhancement of paper properties. *Cellulose Chemistry and Technology*, 53(5–6), 499–507.

Ciancia, M., Matulewicz, M. C., and Tuvikene, R. (2020). Structural Diversity in

Galactans From Red Seaweeds and Its Influence on Rheological Properties. *Frontiers in Plant Science*, 11, 1–19.

Clarke, S. (2003). Aging as war between chemical and biochemical processes: Protein methylation and the recognition of age-damaged proteins for repair. *Ageing Research Reviews*, 2, 263–285.

Craig, A. D., Khattak, F., Hastie, P., Bedford, M. R., and Olukosi, O. A. (2020). Xylanase and xylo-oligosaccharide prebiotic improve the growth performance and concentration of potentially prebiotic oligosaccharides in the ileum of broiler chickens. *British Poultry Science*, Taylor & Francis, 61(1), 70–78.

Cui, D., Yang, J., Lu, B., and Shen, H. (2021). Efficient Preparation of Chitooligosaccharide With a Potential Chitosanase Csn-SH and Its Application for Fungi Disease Protection. *Frontiers in Microbiology*, 12(682829), 1–12.

Cummings, J. H., and Stephen, A. M. (2007). Carbohydrate terminology and classification. *European Journal of Clinical Nutrition*, 61, S5–S18.

Daniel, R. M., Peterson, M. E., Danson, M. J., Price, N. C., Kelly, S. M., Monk, C. R., Weinberg, C. S., Oudshoorn, M. L., and Lee, C. K. (2010). The molecular basis of the effect of temperature on enzyme activity. *Biochemical Journal*, 425, 353–360.

Davies, G., and Henrissat, B. (1995). Structures and mechanisms of glycosyl hydrolases. *Current Biology*, 3(9), 853–859.

Delattre, C., Fenoradosoa, T. A., and Michaud, P. (2011). Galactans: An overview of their most important sourcing and applications as natural polysaccharides. *Brazilian Archives of Biology and Technology*, 54(6), 1075–1092.

Devine, P. N., Howard, R. M., Kumar, R., Thompson, M. P., Truppo, M. D., and Turner, N. J. (2018). Extending the application of biocatalysis to meet the challenges of drug development. *Nature Reviews Chemistry*, Springer US, 2, 409–421.

Dvorak, P., Chrast, L., Nikel, P. I., Fedr, R., Soucek, K., Sedlackova, M., Chaloupkova, R., Lorenzo, V., Prokop, Z., and Damborsky, J. (2015). Exacerbation of substrate toxicity by IPTG in *Escherichia coli* BL21(DE3) carrying a synthetic metabolic pathway. *Microbial Cell Factories*, BioMed Central, 14(201), 1–15.

Faijes, M., Castejón-Vilatersana, M., Val-Cid, C., and Planas, A. (2019). Enzymatic and cell factory approaches to the production of human milk oligosaccharides.

Biotechnology Advances, Elsevier, 37, 667–697.

Fara, A., Sabater, C., Palacios, J., Requena, T., Montilla, A., and Zárate, G. (2020). Prebiotic galactooligosaccharides production from lactose and lactulose by *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL450. *Food and Function*, Royal Society of Chemistry, 11(7), 5875–5886.

Farewell, A., and Neidhardt, F. C. (1998). Effect of temperature on in vivo protein synthetic capacity in *Escherichia coli*. *Journal of Bacteriology*, 180(17), 4704–4710.

Fazaeli, A., Golestani, A., Lakzaei, M., Rasi Varaei, S. S., and Aminian, M. (2019). Expression optimization , purification , and functional characterization of cholesterol oxidase from Chromobacterium sp . DS1. *PLoS ONE*, 14(2), 1–15.

Fazekas, E., Szabó, K., Kandra, L., and Gyémánt, G. (2013). Unexpected mode of action of sweet potato β-amylase on maltooligomer substrates. *Biochimica et Biophysica Acta - Proteins and Proteomics*, Elsevier B.V., 1834, 1976–1981.

Fei, D., Zhang, H., Diao, Q., Jiang, L., Wang, Q., Zhong, Y., Fan, Z., and Ma, M. (2015). Codon optimization, expression in *Escherichia coli*, and immunogenicity of recombinant Chinese sacbrood virus (CSBV) structural proteins VP1, VP2, and VP3. *PLoS ONE*, 10(6), 1–12.

Fischer, C., and Kleinschmidt, T. (2018). Combination of two β-galactosidases during the synthesis of galactooligosaccharides may enhance yield and structural diversity. *Biochemical and Biophysical Research Communications*, Elsevier Ltd, 506(1), 211–215.

Fischer, C., and Kleinschmidt, T. (2021). Synthesis of galactooligosaccharides by Cryptococcus laurentii and *Aspergillus oryzae* using different kinds of acid whey. *International Dairy Journal*, Elsevier Ltd, 112(104867), 1–9.

Frankenberger, W. T., and Johanson, J. B. (1982). Effect of pH on Enzyme Stability in Soils. *Soil Biology and Biochemistry*, 14, 433–437.

Frey, D. D., Engelhardt, F., and Greitzer, E. M. (2003). A role for "one-factor-at-a-time" experimentation in parameter design. *Research in Engineering Design*, 14, 65–74.

Fujita, K., Sakaguchi, T., Sakamoto, A., Shimokawa, M., and Kitahara, K. (2014). *Bifidobacterium longum* subsp. *longum* Exo-beta-1,3-Galactanase, an Enzyme for the Degradation of Type II Arabinogalactan. *Applied and Environmental Microbiology*, 80(15), 4577–4584.

Fujita, K., Sakamoto, A., Kaneko, S., Kotake, T., and Tsumuraya, Y. (2019). Degradative enzymes for type II arabinogalactan side chains in *Bifidobacterium longum* subsp. *longum*. *Applied Microbiology and Biotechnology*, Applied Microbiology and Biotechnology, 103, 1299–1310.

Futami, J., Miyamoto, A., Hagimoto, A., Suzuki, S., Futami, M., and Hiroko, T. (2017). Evaluation of irreversible protein thermal inactivation caused by breakage of disulphide bonds using methanethiosulphonate. *Scientific Reports*, Springer US, 7(12471), 1–10.

Gajdhane, S. B., Bhagwat, P. K., and Dandge, P. B. (2016). Response surface methodology-based optimization of production media and purification of a-galactosidase in solid-state fermentation by Fusarium moniliforme NCIM 1099. *3 Biotech*, Springer Berlin Heidelberg, 6(260), 1–14.

Gasser, B., Saloheimo, M., Rinas, U., Dragosits, M., Rodríguez-Carmona, E., Baumann, K., *et al*. (2008). Protein folding and conformational stress in microbial cells producing recombinant proteins: A host comparative overview. *Microbial Cell Factories*, 7(11), 1–18.

Gawkowska, D., Cybulska, J., and Zdunek, A. (2018). Structure-related gelling of pectins and linking with other natural compounds: A review. *Polymers*, 10(762), 1–25.

González-Ayón, M. A., Licea-Claveríe, Á., Valdez-Torres, J. B., Picos-corrales, L. A., Vélez-de la Rocha, R., Contreras-esquivel, J. C., Labavitch, J. M., and Sañudo-barajas, J. A. (2019). Enzyme-Catalyzed Production of Potato Galactan-Oligosaccharides and Its Optimization by Response Surface Methodology. *Materials*, 12(1465), 1–15.

González-Delgado, I., López-Muñoz, M. J., Morales, G., and Segura, Y. (2016). Optimisation of the synthesis of high galacto-oligosaccharides (GOS) from lactose with β-galactosidase from Kluyveromyces lactis. *International Dairy Journal*, 61, 211–219.

Gowdhaman, D., and Ponnusami, V. (2015). Production and optimization of xylooligosaccharides from corncob by Bacillus aerophilus KGJ2 xylanase and its antioxidant potential. *International Journal of Biological Macromolecules*, Elsevier B.V., 79, 595–600.

Hahor, W., Thongprajukaew, K., and Suanyuk, N. (2019). Effects of dietary supplementation of oligosaccharides on growth performance, gut health and

immune response of hybrid catfish (Pangasianodon gigas × <i>Pangasianodon hypophthalmus). *Aquaculture*, Elsevier, 507(April), 97–107.

Hamidon, N. H., and Zaidel, D. N. A. (2017). Effect of extraction conditions on pectin yield extracted from sweet potato peels residues using hydrochloric acid. *Chemical Engineering Transactions*, 56, 979–984.

Hanson, A. D., McCarty, D. R., Henry, C. S., Xian, X., Joshi, J., Patterson, J. A., García-García, J. D., Fleischmann, S. D., Tivendale, N. D., and Harvey Millar, A. (2021). The number of catalytic cycles in an enzyme's lifetime and why it matters to metabolic engineering. *Proceedings of the National Academy of Sciences of the United States of America*, 118(13), 1–9.

Henrissat, B. (1991). A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochemical Journal*, 280, 309–316.

Hinz, S. W. A., Pastink, M. I., Van Den Broek, L. A. M., Vincken, J. P., and Voragen, A. G. J. (2005). *Bifidobacterium longum* endogalactanase liberates galactotriose from type I galactans. *Applied and Environmental Microbiology*, 71(9), 5501–5510.

Hodoniczky, J., Morris, C. A., and Rae, A. L. (2012). Oral and intestinal digestion of oligosaccharides as potential sweeteners: A systematic evaluation. *Food Chemistry*, Elsevier Ltd, 132, 1951–1958.

Hu, Y., Li, T., Tu, Z., He, Q., Li, Y., and Fu, J. (2020). Engineering a recombination neutral protease I from *Aspergillus oryzae* to improve enzyme activity at acidic pH. *RSC Advances*, Royal Society of Chemistry, 10, 30692–30699.

Huang, S. X., Hou, D. Z., Qi, P. X., Wang, Q., Chen, H. L., Ci, L. Y., and Chen, S. (2020). Enzymatic synthesis of non-digestible oligosaccharide catalyzed by dextransucrase and dextranase from maltose acceptor reaction. *Biochemical and Biophysical Research Communications*, 523, 651–657.

Humbird, D., Davis, R., Tao, L., Kinchin, D., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., and Dudgeon, D. (2011). *Process design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol*. *Technical report NREL/TP-5100-47764*, National Renewable Energy Laboratory, Golden, CO, USA.

Jaafar, N. R., Ahmad, R. A., Nawawi, N. N., Abd Rahman, N. H., Shamsul Annuar, N. A., Rahman, R. A., and Illias, R. M. (2021). Synergistic action of cyclodextrin glucanotransferase and maltogenic amylase improves the bioconversion of starch

to malto-oligosaccharides. *Process Biochemistry*, Elsevier Ltd, 103, 9–17.

Jana, U. K., and Kango, N. (2020). Characteristics and bioactive properties of mannooligosaccharides derived from agro-waste mannans. *International Journal of Biological Macromolecules*, Elsevier B.V., 149, 931–940.

Jernejc, K., and Legiša, M. (2002). The influence of metal ions on malic enzyme activity and lipid synthesis in Aspergillus niger). *FEMS Microbiology Letters*, 217, 185–190.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., and Madden, T. L. (2008). NCBI BLAST: a better web interface. *Nucleic acids research*, 36, 5–9.

Jongkees, S. A. K., and Withers, S. G. (2014). Unusual enzymatic glycoside cleavage mechanisms. *Accounts of Chemical Research*, 47, 226–235.

Kambourova, M. (2018). Thermostable enzymes and polysaccharides produced by thermophilic bacteria isolated from Bulgarian hot springs. *Engineering in Life Sciences*, 18(11), 758–767.

Kazuoka, T., Masuda, Y., Oikawa, T., and Soda, K. (2003). Thermostable aspartase from a marine psychrophile, *Cytophaga* sp. KUC-1: Molecular characterization and primary structure. *Journal of Biochemistry*, 133, 51–58.

Khodaei, N., and Karboune, S. (2013). Extraction and structural characterisation of rhamnogalacturonan I-type pectic polysaccharides from potato cell wall. *Food Chemistry*, 139, 617–623.

Khodaei, N., and Karboune, S. (2016). Enzymatic generation of galactose-rich oligosaccharides/oligomers from potato rhamnogalacturonan I pectic polysaccharides. *Food Chemistry*, Elsevier Ltd, 197, 406–414.

Khodaei, N., and Karboune, S. (2018). Optimization of enzymatic production of prebiotic galacto/galacto(arabino)-oligosaccharides and oligomers from potato rhamnogalacturonan I. *Carbohydrate Polymers*, Elsevier, 181, 1153–1159.

Khodaei, N., Karboune, S., and Orsat, V. (2016). Microwave-assisted alkaline extraction of galactan-rich rhamnogalacturonan I from potato cell wall by-product. *Food Chemistry*, Elsevier Ltd, 190, 495–505.

Kimura, I., Yoshioka, N., and Tajima, S. (1998). Purification and Characterization of an Endo-1,4-β-D-Galactanase from *Aspergillus sojae*. *Journal of Fermentation and Bioengineering*, 85(1), 48–52.

Kokkonen, P., Beier, A., Mazurenko, S., Damborsky, J., Bednar, D., and Prokop, Z.

(2021). Substrate inhibition by the blockage of product release and its control by tunnel engineering. *RSC Chemical Biology*, Royal Society of Chemistry, 2, 645–655.

Koopaei, N. N., Khadiv-Parsi, P., Khoshayand, M. R., Mazlomi, M. A., Kebriaeezadeh, A., Moloudian, H., Solhi, R., and Aminian, M. (2018). Optimization of rPDT fusion protein expression by *Escherichia coli* in pilot scale fermentation: a statistical experimental design approach. *AMB Express*, Springer Berlin Heidelberg, 8(135), 1–9.

Koshland, D. . (1953). Stereochemistry and the mechanism of enzymatic reactions. *Biological Reviews*, 28, 416–436.

Kotake, T., Hirata, N., Degi, Y., Ishiguro, M., Kitazawa, K., Takata, R., Ichinose, H., Kaneko, S., Igarashi, K., Samejima, M., and Tsumuraya, Y. (2011). Endo-beta-1,3-galactanase from Winter Mushroom *Flammulina velutipes*. *The Journal of Biological Chemistry*, 286(31), 27848–27854.

Kotake, T., Kaneko, S., Kubomoto, A., Haque, A., and Kobayashi, H. (2004). Molecular cloning and expression in *Escherichia coli* of a Trichoderma viride endo- β-(1 → 6)-galactanase gene. *Biochemical Journal*, 377, 749–755.

Kuusk, S., and Väljamäe, P. (2017). When substrate inhibits and inhibitor activates: Implications of β-glucosidases. *Biotechnology for Biofuels*, BioMed Central, 10(7), 1–15.

de la Rosa, O., Flores-Gallegos, A. C., Muñíz-Marquez, D., Nobre, C., Contreras-Esquivel, J. C., and Aguilar, C. N. (2019). Fructooligosaccharides production from agro-wastes as alternative low-cost source. *Trends in Food Science and Technology*, Elsevier, 91, 139–146.

Larentis, A. L., Fabiana, J., Quintal, M., Esteves, S., Vareschini, D. T., Vicente, F., Almeida, R. De, Galvão, M., Galler, R., and Medeiros, M. A. (2014). Evaluation of pre-induction temperature, cell growth at induction and IPTG concentration on the expression of a leptospiral protein in *E. coli* using shaking flasks and microbioreactor. *BMC Research Notes*, 7(671), 1–13.

Larsen, D. M., Nyffenegger, C., Swiniarska, M. M., Thygesen, A., Strube, M. L., Meyer, A. S., and Mikkelsen, J. D. (2015). Thermostability enhancement of an endo-1,4-β-galactanase from Talaromyces stipitatus by site-directed mutagenesis. *Applied Microbiology and Biotechnology*, 99, 4245–4253.

Latif, H., Lerman, J. A., Portnoy, V. A., Tarasova, Y., Nagarajan, H., Schrimpe-

Rutledge, A. C., Smith, R. D., Adkins, J. N., Lee, D. H., Qiu, Y., and Zengler, K. (2013). The Genome Organization of *Thermotoga maritima* Reflects Its Lifestyle. *PLoS Genetics*, 9(4).

Lazan, H., Ng, S. Y., Goh, L. Y., and Ali, Z. M. (2004). Papaya β-galactosidase/galactanase isoforms in differential cell wall hydrolysis and fruit softening during ripening. *Plant Physiology and Biochemistry*, 42(11), 847–853.

Lee, J. M., Jang, W. J., Lee, E., and Kong, I. (2020). β-glucooligosaccharides derived from barley β-glucan promote growth of lactic acid bacteria and enhance nisin Z secretion by *Lactococcus lactis*. *LWT - Food Science and Technology*, 122, 109014.

Lee, R. (2019). Statistical Design of Experiments for Screening and Optimization. *Chemie-Ingenieur-Technik*, 91(3), 191–200.

Lee, S., Park, G. G., Jang, J. K., and Park, Y. S. (2018). Optimization of oligosaccharide production from *Leuconostoc lactis* using a response surface methodology and the immunostimulating effects if these oligosaccharides on macrophage cells. *Molecules*, 23, 1–12.

Lemaire, A., Garzon, C. D., Perrin, A., Habrylo, O., Trezel, P., Bassard, S., Lefebvre, V., Wuytswinkel, O. Van, Guillaume, A., Pau-roblot, C., and Pelloux, J. (2020). Three novel rhamnogalacturonan I- pectins degrading enzymes from *Aspergillus aculeatinus*: Biochemical characterization and application potential. *Carbohydrate Polymers*, Elsevier, 248, 116752.

Lever, M. (1973). Calorimetric Determination and Acid Fluorometric with Hydrazide. *Analytical biochemistry*, 7, 274–281.

Li, F., Jin, S., Zhu, L., Zhan, X., Zhao, Y., Liu, L., and Gao, M. (2019). Direct production of curdlan oligosaccharides by coupled fermentation system of Agrobacterium sp.- Pichia pastoris. *The Chinese Journal of Process Engineering*, 19(4), 801–808.

Li, J., Amatuni, A., and Renata, H. (2020). Recent advances in the chemoenzymatic synthesis of bioactive natural products. *Current Opinion in Chemical Biology*, Elsevier Ltd, 55, 111–118.

Li, Y., Zhang, Z., Jing, Y., Ge, X., Wang, Y., Lu, C., Zhou, X., and Zhang, Q. (2017). Statistical optimization of simultaneous saccharification fermentative hydrogen production from Platanus orientalis leaves by photosynthetic bacteria HAU-M1. *International Journal of Hydrogen Energy*, Elsevier Ltd, 42, 5804–5811.

Li, Z., and Rinas, U. (2021). Recombinant protein production-associated metabolic burden reflects anabolic constraints and reveals similarities to a carbon overfeeding response. *Biotechnology and Bioengineering*, 118, 94–105.

de Lima, E. A., Machado, C. B., Sato, H. H., and Ruller, R. (2016). GH53 Endo-Beta-1,4-Galactanase from a Newly Isolated *Bacillus licheniformis* CBMAI 1609 as an Enzymatic Cocktail Supplement for Biomass Saccharification. *Applied Biochemistry and Biotechnology*, 179, 415–426.

Lineweaver, H., and Burk, D. (1934). The Determination of Enzyme Dissociation Constants. *Journal of the American Chemical Society*, 56(3), 658–666.

Ling, N. X., Lee, J., Ellis, M., Liao, M., Mau, S., Guest, D., Bacic, A., Pettolino, F. A., Janssen, P. H., and Kovác, P. (2012). An exo-beta-(1-->3)-D-galactanase from Streptomyces sp. provides insights into type II arabinogalactan structure. *Carbohydrate Research*, 352, 70–81.

Liu, J., Wang, J., Leung, C., and Gao, F. (2018). A multi-parameter optimization model for the evaluation of shale gas recovery enhancement. *Energies*, 11(654), 1–29.

Liu, P., Xie, J., Liu, J., and Ouyang, J. (2019). A novel thermostable β-galactosidase from *Bacillus coagulans* with excellent hydrolysis ability for lactose in whey. *Journal of Dairy Science*, American Dairy Science Association, 102(11), 9740–9748.

Liu, S., Duan, X., Lu, X., and Gao, P. (2006). A novel thermophilic endoglucanase from a mesophilic fungus *Fusarium oxysporum*. *Chinese Science Bulletin*, 51(2), 191–197.

Lombard, V., Ramulu, G. H., Drula, E., Coutinho, P. M., and Henrissat, B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Research*, 42, 490–495.

Love, K. R., and Seeberger, P. H. (2004). Automated Solid-Phase Synthesis of Protected Tumor-Associated Antigen and Blood Group Determinant Oligosaccharides. *Angewandte Chemie - International Edition*, 43, 602–605.

Lu, Z. Y., Feng, L., Jiang, W. D., Wu, P., Liu, Y., Kuang, S. Y., Tang, L., and Zhou, X. Q. (2020). Mannan oligosaccharides improved growth performance and antioxidant capacity in the intestine of on-growing grass carp (Ctenopharyngodon idella). *Aquaculture Reports*, Elsevier, 17, 100313.

Lundemo, P., Karlsson, E. N., and Adlercreutz, P. (2017). Eliminating hydrolytic

activity without affecting the transglycosylation of a GH1 β-glucosidase. *Applied Microbiology and Biotechnology*, Applied Microbiology and Biotechnology, 101, 1121–1131.

Ma, F., Xie, Y., Luo, M., Wang, S., Hu, Y., Liu, Y., Feng, Y., and Yang, G. Y. (2016). Sequence homolog-based molecular engineering for shifting the enzymatic pH optimum. *Synthetic and Systems Biotechnology*, The Authors, 1, 195–206.

Madhuprakash, J., Tanneeru, K., Purushotham, P., Guruprasad, L., and Podile, A. R. (2012). Transglycosylation by Chitinase D from *Serratia proteamaculans* improved through altered substrate interactions. *Journal of Biological Chemistry*, Â© 2012 ASBMB. Currently published by Elsevier Inc; originally published by American Society for Biochemistry and Molecular Biology., 287(53), 44619–44627.

Mallek-Fakhfakh, H., and Belghith, H. (2016). Physicochemical properties of thermotolerant extracellular β-glucosidase from <I>Talaromyces thermophilus</I> and enzymatic synthesis of cello-oligosaccharides. *Carbohydrate Research*, Elsevier Ltd, 419, 41–50.

Maloney, K. P., Truong, V., and Allen, J. C. (2014). Susceptibility of sweet potato (*Ipomoea batatas*) peel proteins to digestive enzymes. *Food Science & Nutrition*, 2(4), 351–360.

Manas, N. H. A., Bakar, F. D. A., and Illias, R. M. (2016). Computational docking, molecular dynamics simulation and subsite structure analysis of a maltogenic amylase from *Bacillus lehensis* G1 provide insights into substrate and product specificity. *Journal of Molecular Graphics and Modelling*, Elsevier Inc., 67, 1–13.

Martín, C., de Moraes Rocha, G. J., dos Santos, J. R. A., Wanderly, de A., and Gouveia, E. R. (2012). Enzyme Loading Dependence of Cellulose Hydrolysis of Sugarcane Bagasse. *Química Nova*, 35(10), 1927–1930.

Martins, G. N., Ureta, M. M., Tymczyszyn, E. E., Castilho, P. C., and Gomez-zavaglia, A. (2019). Technological Aspects of the Production of Fructo and Galacto-oligosachharides. Enzymatic Synthesis and Hydrolysis. *Frontiers in Nutrition*, 6(78).

McArthur, J. B., and Chen, X. (2016). Glycosyltransferase engineering for carbohydrate synthesis. *Biochemical Society Transactions*, 44(1), 129–142.

Mei, X., Tai Hua, M., and Jun Juan, H. (2010). Composition and Physicochemical

Properties of Dietary Fiber Extracted from Residues of 10 Varieties of Sweet Potato by a Sieving Method. *Journal of Agricultural and Food Chemistry*, 58, 7305–7310.

Michalak, M., Thomassen, L. V., Roytio, H., Ouwehand, A. C., Meyer, A. S., and Mikkelsen, J. D. (2012). Expression and characterization of an endo-1,4-β-galactanase from Emericella nidulans in *Pichia pastoris* for enzymatic design of potentially prebiotic oligosaccharides from potato galactans. *Enzyme and Microbial Technology*, Elsevier Inc., 50, 121–129.

Michel, G., Chantalat, L., Duee, E., Barbeyron, T., Henrissat, B., Kloareg, B., and Dideberg, O. (2001). The κ-carrageenase of P. carrageenovora Features a Tunnel-Shaped Active Site: A Novel Insight in the Evolution of Clan-B Glycoside Hydrolases. *Structure*, 9, 513–525.

Misson, M., Jin, B., and Zhang, H. (2020). Interfacial Biocatalytic Performance of Nanofiber-Supported β-Galactosidase for Production of Galacto-Oligosaccharides. *Catalysts*, 479, 1–12.

Miyawaki, O., Kanazawa, T., Maruyama, C., and Dozen, M. (2017). Static and dynamic half-life and lifetime molecular turnover of enzymes. *Journal of Bioscience and Bioengineering*, Elsevier Ltd, 123(1), 28–32.

Mohanraj, R. (2019). *Sweet Potato: Bioactive Compounds and Health Benefits*. *Reference Series in Phytochemistry*, (J. M. Mérillon and K. Ramawat, eds.), Springer, Cham.

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology*, 11, 266–277.

Mohtar, N. S., Rahman, M. B., Rahman, R. N. Z. R. A., Leow, T. C., Salleh, A. B., and Isa, M. N. M. (2016). Expression and characterization of thermostable glycogen branching enzyme from *Geobacillus mahadia* Geo-05. *PeerJ*, 1–12.

Muderspach, S. J., Fredslund, F., Volf, V., Poulsen, J. C. N., Blicher, T. H., Clausen, M. H., Rasmussen, K. K., Krogh, K. B. R. M., Jensen, K., and Lo Leggio, L. (2021). Engineering the substrate binding site of the hyperthermostable archaeal endo-β-1,4-galactanase from *Ignisphaera aggregans*. *Biotechnology for Biofuels*, BioMed Central, 14(183), 1–16.

Mühlmann, M., Forsten, E., Noack, S., and Büchs, J. (2017). Optimizing recombinant protein expression via automated induction profiling in microtiter plates at different temperatures. *Microbial Cell Factories*, BioMed Central, 16(220), 1–

12.

Mussatto, S. I., and Mancilha, I. M. (2007). Non-digestible oligosaccharides: A review. *Carbohydrate Polymers*, 68, 587–597.

Mustafa, A., Buentello, A., Gatlin, D., Lightner, D., Lawrence, A., Mustafa, A., Buentello, A., Gatlin, D., and Lightner, D. (2019). Dietary supplementation of galactooligosaccharides (GOS) in Pacific white shrimp, Litopenaeus vannamei, cultured in a recirculating system and its effects on gut microflora, growth, stress, and immune response. *Journal of Immunoassay and Immunochemistry*, Taylor & Francis, 1–14.

Nakamura, A. N., Maeda, H. M., Mizuno, M. M., Koshi, Y. K., and Nagamatsu, Y. N. (2003). Beta-Galactosidase and Its Significance in Ripening of "'Saijyo'" *Japanese Persimmon* Fruit. *Bioscience, Biotechnology, and Biochemistry*, 67(1), 68–76.

Naushad, M., ALOthman, Z. A., Khan, A. B., and Ali, M. (2012). Effect of ionic liquid on activity, stability, and structure of enzymes: A review. *International Journal of Biological Macromolecules*, Elsevier B.V., 51, 555–560.

Newman, R. H., Vaidya, A. A., Imroz Sohel, M., and Jack, M. W. (2013). Optimizing the enzyme loading and incubation time in enzymatic hydrolysis of lignocellulosic substrates. *Bioresource Technology*, 129, 33–38.

Nguyen, H. H., Park, J., Park, S. J., Lee, C. S., Hwang, S., Shin, Y. B., Ha, T. H., and Kim, M. (2018). Long-term stability and integrity of plasmid-based DNA data storage. *Polymers*, 10(28).

Nobre, C., Filho, E. G. A., Fernandes, F. A. N., Brito, E. S., Rodrigues, S., Teixeira, J. A., and Rodrigues, L. R. (2018). Production of fructo-oligosaccharides by Aspergillus ibericus and their chemical characterization. *LWT - Food Science and Technology*, Elsevier, 89, 58–64.

Noi, N. Van, and Chung, Y. (2017). Optimization of expression and purification of recombinant S1 domain of the porcine epidemic diarrhea virus spike (PEDV- S1) protein in *Escherichia coli*. *Biotechnology & Biotechnological Equipment*, Taylor & Francis, 31(3), 619–629.

Le Nours, J., De Maria, L., Welner, D., Jørgensen, C. T., Christensen, L. L. H., Borchert, T. V, Larsen, S., and Lo Leggio, L. (2008). Investigating the binding of beta-1,4-galactan to *Bacillus licheniformis* beta-1,4-galactanase by crystallography and computational modeling ´. *Proteins*, 75, 977–989.

Le Nours, J., Ryttersgaard, C., Lo Leggio, L., Østergaard, P. R., Borchert, T. V., Christensen, L. L. H., and Larsen, S. (2003). Structure of two fungal β-1,4-galactanases: Searching for the basis for temperature and pH optimum. *Protein Science*, 12, 1195–1204.

Nurdjanah, S. (2008). Extraction And Characterisation Of Pectin From Australian And Indonesian Sweet Potato (Ipomoea batatas L .). *PhD. Thesis*, University of New South Wales.

O'Connell Motherway, M., Kinsella, M., Fitzgerald, G. F., and Van Sinderen, D. (2013). Transcriptional and functional characterization of genetic elements involved in galacto-oligosaccharide utilization by *Bifidobacterium breve* UCC2003. *Microbial Biotechnology*, 6(1), 67–79.

Ojwach, J., Kumar, A., Mukaratirwa, S., and Mutanda, T. (2020). Fructooligosaccharides synthesized by fructosyltransferase from an indigenous coprophilous Aspergillus niger strain XOBP48 exhibits antioxidant activity. *Bioactive Carbohydrates and Dietary Fibre*, Elsevier Ltd, 24, 100238.

Okawa, M., Fukamachi, K., and Tanaka, H. (2013). Identification of an exo-ß-1,3-D-galactanase from *Fusarium oxysporum* and the synergistic effect with related enzymes on degradation of type II arabinogalactan. *Applied Microbiology and Biotechnology*, 97, 9685–9694.

Okemoto, K., Uekita, T., Tsumuraya, Y., Hashimoto, Y., and Kasama, T. (2003). Purification and characterization of an endo-beta- (1-->6)-galactanase from *Trichoderma viride*. *Carbohydrate Research*, 338, 219–230.

Onyeogaziri, F. C., and Papaneophytou, C. (2019). A General Guide for the Optimization of Enzyme Assay Conditions Using the Design of Experiments Approach. *SLAS Discovery*, 24(5), 587–596.

Pace, C. N., Grimsley, G. R., and Scholtz, J. M. (2009). Protein Ionizable Groups: pK Values and Their Contribution to Protein Stability and Solubility. *Journal of Biological Chemistry*, 284(20), 13285–13289.

Panza, M., Pistorio, S. G., Stine, K. J., and Demchenko, A. V. (2018). Automated Chemical Oligosaccharide Synthesis: Novel Approach to Traditional Challenges. *Chemical Reviews*, 118(17), 8105–8150.

Papaneophytou, C., and Kontopidis, G. (2016). A comparison of statistical approaches used for the optimization of soluble protein expression in *Escherichia coli*. *Protein Expression and Purification*, Elsevier Ltd, 120, 126–137.

Park, A. R., and Oh, D. K. (2010). Galacto-oligosaccharide production using microbial β-galactosidase: Current state and perspectives. *Applied Microbiology and Biotechnology*, 85(5), 1279–1286.

Penksza, P., Juhász, R., Szabó-Nótin, B., and Sipos, L. (2020). Xylo-oligosaccharides as texture modifier compounds in aqueous media and in combination with food thickeners. *Food Science and Nutrition*, 8, 3023–3030.

Perugino, G., Trincone, A., Rossi, M., and Moracci, M. (2004). Oligosaccharide synthesis by glycosynthases. *Trends in Biotechnology*, 22(1), 31–37.

Petersen, T. N., Brunak, S., Von Heijne, G., and Nielsen, H. (2011). SignalP 4.0: Discriminating signal peptides from transmembrane regions. *Nature Methods*, Nature Publishing Group, 8(10), 785–786.

Piubelli, L., Campa, M., Temporini, C., Binda, E., Mangione, F., Amicosante, M., Terreni, M., Marinelli, F., and Pollegioni, L. (2013). Optimizing *Escherichia coli* as a protein expression platform to produce *Mycobacterium tuberculosis* immunogenic proteins. *Microbial Cell Factories*, 12(115), 1–14.

Piva, L. C., Bentacur, M. O., Reis, V. C. B., De Marco, J. L., Moraes, L. M. P. de, and Torres, F. A. G. (2017). Molecular strategies to increase the levels of heterologous transcripts in *Komagataella phaffii* for protein production. *Bioengineered*, Taylor & Francis, 8(5), 441–445.

Polic, V., and Auclair, K. (2014). Controlling substrate specificity and product regio- and stereo-selectivities of P450 enzymes without mutagenesis. *Bioorganic and Medicinal Chemistry*, Elsevier Ltd, 22, 5547–5554.

Prejanò, M., Alberto, M. E., Russo, N., Toscano, M., and Marino, T. (2020). The effects of the metal ion substitution into the active site of metalloenzymes: A theoretical insight on some selected cases. *Catalysts*, 10(1038), 1–28.

Qian, J., Zhang, H., and Liao, Q. (2011). The properties and kinetics of enzymatic reaction in the process of the enzymatic extraction of fish oil. *Journal of Food Science and Technology*, 48(3), 280–284.

Qing, G., Ma, L. C., Khorchid, A., Swapna, G. V. T., Mal, T. K., Takayama, M. M., Xia, B., Phadtare, S., Ke, H., Acton, T., Montelione, G. T., Ikura, M., and Inouye, M. (2004). Cold-shock induced high-yield protein production in *Escherichia coli*. *Nature Biotechnology*, 22(7), 877–882.

Raj, A. A. S., Rubila, S., Jayabalan, R., and Ranganathan, T. . (2012). A Review on Pectin: Chemistry due to General Properties of Pectin and its Pharmaceutical

Uses. *Open Access Scientific Reports*, 1(12), 1–4.

Rajulapati, V., Dhillon, A., and Goyal, A. (2021). Enzymatically produced pectic-oligosaccharides from fruit waste of *Citrus reticulata* (mandarin) peels display cytotoxicity against colon cancer cells. *Bioresource Technology Reports*, Elsevier Ltd, 15, 100740.

Ratner, B. (2009). The correlation coefficient: Its values range between + 1/ − 1, or do they? *Journal of Targeting, Measurement and Analysis for Marketing*, 17(2), 139–142.

Reed, M. C., Lieb, A., and Nijhout, H. F. (2010). The biological significance of substrate inhibition: A mechanism with diverse functions. *Bioessays*, 32, 422–429.

Reichembach, L. H., and de Oliveira Petkowicz, L. C. (2021). Pectins from alternative sources and uses beyond sweets and jellies: An overview. *Food Hydrocolloids*, Elsevier Ltd, 118(106824), 1–20.

Rezaei, K., Jenab, E., and Temelli, F. (2007). Effects of water on enzyme performance with an emphasis on the reactions in supercritical fluids. *Critical Reviews in Biotechnology*, 27, 183–195.

Ribeiro, A. J. M., Tyzack, J. D., Borkakoti, N., Holliday, G. L., and Thornton, J. M. (2020). A global analysis of function and conservation of catalytic. *Journal of Biological Chemistry*, 295(2), 314–324.

Rudroff, F., Mihovilovic, M. D., Gröger, H., Snajdrova, R., Iding, H., and Bornscheuer, U. T. (2018). Opportunities and challenges for combining chemo- and biocatalysis. *Nature Catalysis*, Springer US, 1, 12–22.

Ryttersgaard, C., Leggio, L. Lo, Coutinho, P. M., Henrissat, B., and Larsen, S. (2002). *Aspergillus aculeatus* beta-1,4-Galactanase : Substrate Recognition and Relations to Other Glycoside Hydrolases in Clan GH-A. *Biochemistry*, 41, 15135–15143.

Ryttersgaard, C., Le Nours, J., Lo Leggio, L., Jørgensen, C. T., Christensen, L. L. H., Bjørnvad, M., and Larsen, S. (2004). The Structure of Endo-beta-1,4-galactanase from *Bacillus licheniformis* in Complex with Two Oligosaccharide Products. *Journal of Molecular Biology*, 341, 107–117.

Sabbavarapu, N. M., and Seeberger, P. H. (2021). Automated Glycan Assembly of Oligogalactofuranosides Reveals the Influence of Protecting Groups on Oligosaccharide Stability. *Journal of Organic Chemistry*, 86, 7280–7287.

Saito, Y., Kitagawa, W., Kumagai, T., Tajima, N., Nishimiya, Y., Tamano, K.,

Yasutake, Y., Tamura, T., and Kameda, T. (2019). Developing a codon optimization method for improved expression of recombinant proteins in actinobacteria. *Scientific Reports*, Springer US, 9(8338), 1–10.

Sajib, M., Albers, E., Langeland, M., and Undeland, I. (2020). Understanding the effect of temperature and time on protein degree of hydrolysis and lipid oxidation during ensilaging of herring (*Clupea harengus*) filleting co-products. *Scientific Reports*, 10, 1–13.

Sakamoto, T., Nishimura, Y., and Makino, Y. (2013). Biochemical characterization of a GH53 endo-β-1,4-galactanase and a GH35 exo-β-1,4-galactanase from *Penicillium chrysogenum*. *Applied Microbiology and Biotechnology*, 97, 2895–2906.

Samanta, A. K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A. P., Senani, S., and Sridhar, M. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. *Bioactive Carbohydrates and Dietary Fibre*, Elsevier, 62–71.

Samanta, A. K., Jayapal, N., Kolte, A. P., Senani, S., Sridhar, M., Suresh, K. P., and Sampath, K. T. (2012). Enzymatic production of xylooligosaccharides from alkali solubilized xylan of natural grass (*Sehima nervosum*). *Bioresource Technology*, Elsevier Ltd, 112, 199–205.

San-miguel, T., Pérez-bermúdez, P., and Gavidia, I. (2013). Production of soluble eukaryotic recombinant proteins in *E . coli* is favoured in early log-phase cultures induced at low temperature. *SpringerPlus*, 2(89), 2–5.

Van Der Sanden, M. J. T., Nagy, K., Semsei, I., and Zs.-Nagy, I. (1995). An in vitro model of aging: the influence of increasing physical density on enzyme activities of trypsin, xanthine oxidase and superoxide dismutase. *Archives of Gerontology and Geriatrics*, 20, 273–282.

dos Santos, A. M. P., Lima, J. S., dos Santos, Ivanice, F., Silva, E. F. R., de Santana, F. A., de Araujo, D. G. G. R., and dos Santos, L. O. (2019). Mineral and centesimal composition evaluation of conventional and organic cultivars sweet potato (*Ipomoea batatas* (L .) Lam) using chemometric tools. *Food Chemistry*, Elsevier, 273, 166–171.

Seeberger, P. H., and Werz, D. B. (2007). Synthesis and medical applications of oligosaccharides. *Nature*, 446, 1046–1051.

Shahi, Z., Sayyed-alangi, S. Z., and Naja, L. (2020). Effects of enzyme type and

process time on hydrolysis degree, electrophoresis bands and antioxidant properties of hydrolyzed proteins derived from defatted *Bunium persicum Bioss* press cake. *Heliyon*, 6, e03365.

Shang, Y., Kumar, S., Thippareddi, H., and Kim, W. K. (2018). Effect of dietary fructooligosaccharide (FOS) supplementation on ileal microbiota in broiler chickens. *Poultry Science*, Poultry Science Association Inc., 97(10), 3622–3634.

Sharma, K., Khaire, K. C., Thakur, A., Moholkar, V. S., Goyal, A., and Goyal, A. (2020). Acacia Xylan as a Substitute for Commercially Available Xylan and Its Application in the Production of Xylooligosaccharides. *ACS Omega*, 5(23), 13729–13738.

Shi, P., Chen, X., Meng, K., Huang, H., Bai, Y., Luo, H., Yang, P., and Yao, B. (2013). Distinct actions by *Paenibacillus* sp. strain E18 αlpha-L-arabinofuranosidases and xylanase in xylan degradation. *Applied and Environmental Microbiology*, 79(6), 1990–1995.

Show, P. L., Oladele, K. O., Siew, Q. Y., Aziz Zakry, F. A., Lan, J. C. W., and Ling, T. C. (2015). Overview of citric acid production from *Aspergillus niger*. *Frontiers in Life Science*, 8(3), 271–283.

Siddiqui, M. F., and Bano, B. (2018). Insight into the functional and structural transition of garlic phytocystatin induced by urea and guanidine hydrochloride: A comparative biophysical study. *International Journal of Biological Macromolecules*, Elsevier B.V., 106, 20–29.

Singh, R. D., Nadar, C. G., Muir, J., and Arora, A. (2019). Green and clean process to obtain low degree of polymerisation xylooligosaccharides from almond shell. *Journal of Cleaner Production*, Elsevier Ltd, 241(118237), 1–9.

Smith, J. D., Richardson, N. E., and Robinson, A. S. (2005). Elevated expression temperature in a mesophilic host results in increased secretion of a hyperthermophilic enzyme and decreased cell stress. *Biochimica et Biophysica Acta 1752*, 18–25.

Soga, T., and Heiger, D. N. (1998). Simultaneous determination of monosaccharides in glycoproteins by capillary electrophoresis. *Analytical biochemistry*, 261, 73–78.

Sørlie, M., Horn, S. J., Vaaje-Kolstad, G., and Eijsink, V. G. H. (2020). Using chitosan to understand chitinases and the role of processivity in the degradation of recalcitrant polysaccharides. *Reactive and Functional Polymers*, Elsevier,

148(104488), 1–11.

Sousa, S. F., Ramos, M. J., Lim, C., and Fernandes, P. A. (2015). Relationship between Enzyme / Substrate Properties and Enzyme Efficiency in Hydrolases. *ACS Catalysis*, 5(10), 5877–5887.

Strazzulli, A., Cobucci-Ponzano, B., Carillo, S., Bedini, E., Corsaro, M. M., Pocsfalvi, G., Withers, S. G., Rossi, M., and Moracci, M. (2017). Introducing transgalactosylation activity into a family 42 β-galactosidase. *Glycobiology*, 27(5), 425–437.

Sun, L., Ropartz, D., Cui, L., Shi, H., Ralet, M. C., and Zhou, Y. (2019). Structural characterization of rhamnogalacturonan domains from Panax ginseng C. A. Meyer. *Carbohydrate Polymers*, Elsevier, 203, 119–127.

Surek, E., and Buyukkileci, A. O. (2017). Production of xylooligosaccharides by autohydrolysis of hazelnut (*Corylus avellana* L.) shell. *Carbohydrate Polymers*, Elsevier Ltd., 174, 565–571.

Tabachnikov, O., and Shoham, Y. (2013). Functional characterization of the galactan utilization system of *Geobacillus stearothermophilus*. *FEBS Journal*, 280(3), 950–964.

Takamine, K., Abe, J., Shimono, K., Sameshima, Y., Morimura, S., and Kida, K. (2007). Physicochemical and Gelling Characterizations of Pectin Extracted from Sweet POtato Pulp. *Journal of Applied Glycoscience*, 54, 211–216.

Takata, R., Tokita, K., Mori, S., Shimoda, R., Harada, N., and Ichinose, H. (2010). Degradation of carbohydrate moieties of arabinogalactan-proteins by glycoside hydrolases from Neurospora crassa,. *Carbohydrate Research*, Elsevier Ltd, 345, 2516–2522.

Tavares, F., Silva, E. A. Da, Pinzan, F., Canevesi, R. S., Milinsk, M. C., Scheufele, F. B., Borba, C. E., Tavares, F., Silva, E. A. Da, Pinzan, F., Rafael, S., Milinsk, M. C., Scheufele, F. B., and Borba, C. E. (2018). Hydrolysis of crambe oil by enzymatic catalysis: An evaluation of the operational conditions. *Biocatalysis and Biotransformation*, Informa UK Limited, trading as Taylor & Francis Group, 1–14.

Teze, D., Hendrickx, J., Czjzek, M., Ropartz, D., Sanejouand, Y., Tran, V., Tellier, C., and Dion, M. (2014). Semi-rational approach for converting a GH1 beta-glycosidase into a beta-transglycosidase. *Protein Engineering, Design & Selection*, 27(1), 13–19.

Tian, J., Yan, Y., Yue, Q., Liu, X., Chu, X., Wu, N., and Fan, Y. (2017). Predicting synonymous codon usage and optimizing the heterologous gene for expression in *E. coli*. *Scientific Reports*, Springer US, 7(9926), 1–9.

Tingirikari, J. M. R. (2018). Microbiota-accessible pectic poly- and oligosaccharides in gut health. *Food and Function*, Royal Society of Chemistry, 9, 5059–5073.

Tomita, S., and Shiraki, K. (2011). Why do solution additives suppress the heat-induced inactivation of proteins? inhibition of chemical modifications. *Biotechnology Progress*, 27(3), 855–862.

Torpenholt, S., Le, J., Christensen, U., Jahn, M., Withers, S., Rahbek, P., Borchert, T. V, Poulsen, J., and Lo, L. (2011). Activity of three beta-1,4-galactanases on small chromogenic substrates. *Carbohydrate Research*, 346, 2028–2033.

Torpenholt, S., N. Poulsen, J.-C., Muderspach, S. J., Maria, L. De, and Leggio, L. Lo. (2019). Structure of *Aspergillus aculeatus* beta-1,4-galactanase in complex with galactobiose. *Structural Biology Communications*, International Union of Crystallography, F75, 399–404.

Trösemeier, J. H., Rudorf, S., Loessner, H., Hofner, B., Reuter, A., Schulenborg, T., Koch, I., Bekeredjian-Ding, I., Lipowsky, R., and Kamp, C. (2019). Optimizing the dynamics of protein expression. *Scientific Reports*, 9, 1–15.

Tsumura, K., Hashimoto, Y., Akiba, T., and Horikoshi, K. (1991). Purifications and Properties of Galactanases from Alkalophilic *Bacillus* sp. S-2 and S-39. *Agricultural and Biological Chemistry*, 55(5), 1265–1271.

Urban, A., Ansmant, I., and Motorin, Y. (2003). Optimisation of expression and purification of the recombinant Yol066 (Rib2) protein from *Saccharomyces cerevisiae*. *Journal of Chromatography B*, 786, 187–195.

Ureta, M. M., Romano, N., Kakisu, E., and Gómez-Zavaglia, A. (2019). Synthesis of fructo-oligosaccharides using grape must and sucrose as raw materials. *Food Research International*, Elsevier, 123, 166–171.

Vera, C., Guerrero, C., Conejeros, R., and Illanes, A. (2012). Synthesis of galacto-oligosaccharides by β-galactosidase from *Aspergillus oryzae* using partially dissolved and supersaturated solution of lactose. *Enzyme and Microbial Technology*, Elsevier Inc., 50(3), 188–194.

Voragen, A. G. J., Coenen, G. J., Verhoef, R. P., and Schols, H. A. (2009). Pectin, a versatile polysaccharide present in plant cell walls. *Structural Chemistry*, 20, 263–275.

Waegeman, H., and Soetaert, W. (2011). Increasing recombinant protein production in *Escherichia coli* through metabolic and genetic engineering. *Journal of Industrial Microbiology and Biotechnology*, 38, 1891–1910.

Walsh, C., Lane, J. A., van Sinderen, D., and Hickey, R. M. (2020). From lab bench to formulated ingredient: Characterization, production, and commercialization of human milk oligosaccharides. *Journal of Functional Foods*, Elsevier, 72, 104052.

Wan Azelee, N. I., Jahim, J. M., Ismail, A. F., Fuzi, S. F. Z. M., Rahman, R. A., and Md Illias, R. (2016). High xylooligosaccharides (XOS) production from pretreated kenaf stem by enzyme mixture hydrolysis. *Industrial Crops and Products*, Elsevier B.V., 81, 11–19.

Wang, Q., Cen, Z., and Zhao, J. (2015). The survival mechanisms of thermophiles at high temperatures: An angle of omics. *Physiology*, 30, 97–106.

Wen, L., Edmunds, G., Gibbons, C., Zhang, J., Gadi, M. R., Zhu, H., Fang, J., Liu, X., Kong, Y., and Wang, P. G. (2018). Toward Automated Enzymatic Synthesis of Oligosaccharides. *Chemical Reviews*, review-article, American Chemical Society, 118, 8151–8187.

Wilkowska, A., Nowak, A., Antczak-Chrobot, A., Motyl, I., Czy, A., and Paliwoda, A. (2019). Structurally different pectic oligosaccharides produced from apple pomace and their biological activity in vitro. *Foods*, 8(365), 1–22.

Woodley, J. M. (2006). Choice of biocatalyst form for scalable processes. *Biocatalysis*, 34, 301–303.

Wu, J., Yang, Z., Yang, X., Chen, X., Zhang, H., and Zhan, X. (2021). Synthesis of branched β-1,3-glucan oligosaccharide with narrow degree of polymerization by fungi co-cultivation. *Carbohydrate Polymers*, Elsevier Ltd, 273, 118582.

Xiao, Z., Guo, Y., Liu, Y., Li, L., Zhang, Q., Wen, L., Wang, X., Kondengaden, S. M., Wu, Z., Zhou, J., Cao, X., Li, X., Ma, C., and Wang, P. G. (2016). Chemoenzymatic Synthesis of a Library of Human Milk Oligosaccharides. *Journal of Organic Chemistry*, 81(14), 5851–5865.

Yang, H., Ichinose, H., Yoshida, M., Nakajima, M., Kobayashi, H., and Kaneko, S. (2006). Characterization of a thermostable endo-β-1,4-D-galactanase from the hyperthermophile *Thermotoga maritima*. *Bioscience, Biotechnology, and Biochemistry*, 70(2), 538–541.

Yang, J., and Han, Z. (2018). Understanding the positional binding and substrate interaction of a highly thermostable GH10 xylanase from Thermotoga maritima

by molecular docking. *Biomolecules*, 8(64), 1–14.

Yang, J., Mu, T., and Ma, M. (2018). Extraction, structure, and emulsifying properties of pectin from potato pulp. *Food Chemistry*, Elsevier, 244, 197–205.

Yao, W., Yan, J., Chen, X., Wang, F., and Cao, H. (2015). Chemoenzymatic synthesis of lacto-N-tetrasaccharide and sialyl lacto-N-tetrasaccharides. *Carbohydrate Research*, Elsevier Ltd, 401, 5–10.

Yi, G., Thon, M. R., and Sze, S. H. (2012). Supervised protein family classification and new family construction. *Journal of Computational Biology*, 19(8), 957–967.

Yin, H., Bultema, J. B., Dijkhuizen, L., and van Leeuwen, S. S. (2017a). Reaction kinetics and galactooligosaccharide product profiles of the β-galactosidases from *Bacillus circulans, Kluyveromyces lactis* and *Aspergillus oryzae*. *Food Chemistry*, Elsevier Ltd, 225, 230–238.

Yin, H., Pijning, T., Meng, X., Dijkhuizen, L., and van Leeuwen, S. S. (2017b). Biochemical Characterization of the Functional Roles of Residues in the Active Site of the β-Galactosidase from *Bacillus circulans* ATCC 31382. *Biochemistry*, 56(24), 3109–3118.

Yoshimi, Y., Yaguchi, K., Kaneko, S., and Tsumuraya, Y. (2017). Properties of two fungal endo-beta-1,3-galactanases and their synergistic action with an exo-beta-1,3-galactanase in degrading arabinogalactan-proteins. *Carbohydrate Research*, Elsevier Ltd, 453–454, 26–35.

Yu, L., and O'Sullivan, D. J. (2014). Production of galactooligosaccharides using a hyperthermophilic β-galactosidase in permeabilized whole cells of *Lactococcus lactis*. *Journal of Dairy Science*, 97(2), 694–703.

Zaidel, D. N. A., Zainudin, N. N., Jusoh, Y. M. M., and Muhamad, I. I. (2015). Extraction and characterisation of pectin from sweet potato (*Ipomoea Batatas*) pulp. *Journal of Engineering Science and Technology*, (3), 22–29.

Zając, G., Szyszlak-Bargłowicz, J., Gołębiowski, W., and Szczepanik, M. (2018). Chemical characteristics of biomass ashes. *Energies*, 11(2885), 1–15.

Zavaleta, V., and Eyzaguirre, J. (2016). *Penicillium purpurogenum* produces a highly stable endo-β-(1,4)-galactanase. *Applied Biochemistry and Biotechnology*, 180, 1313–1327.

Zhang, W., Lu, J., Zhang, S., Liu, L., Pang, X., and Lv, J. (2018). Development an effective system to expression recombinant protein in *E. coli* via comparison and optimization of signal peptides: Expression of *Pseudomonas fluorescens* BJ-10

thermostable lipase as case study. *Microbial Cell Factories*, BioMed Central, 17(50), 1–12.

Zhang, W., Ray, C., Poojary, M. M., Jansson, T., Olsen, K., and Lund, M. N. (2019). Inhibition of Maillard Reactions by Replacing Galactose with Galacto-Oligosaccharides in Casein Model Systems. *Journal of Agricultural and Food Chemistry*, 67(3), 875–886.

Zhang, Y., Mu, T., and Zhang, M. (2013). Optimisation of acid extraction of pectin from sweet potato residues by response surface methodology and its antiproliferation effect on cancer cells. *International of Food Sciences & Technology*, 48, 778–785.

Zhao, J., Zhang, F., Liu, X., St, K., and Zhang, A. (2017). Isolation of a lectin binding rhamnogalacturonan-I containing pectic polysaccharide from pumpkin. *Carbohydrate Polymers*, Elsevier Ltd., 163, 330–336.

Zheng, F., Vermaas, J. V., Zheng, J., Wang, Y., Tu, T., Wang, X., Xie, X., Yao, B., Beckham, G. T., and Luo, H. (2018). Activity and thermostability of GH5 endoglucanase chimeras from mesophilic and thermophilic parents. *Applied and Environmental Microbiology*, 85(5), 1–18.

Zhong, C., Ukowitz, C., Domig, K. J., and Nidetzky, B. (2020). Short-Chain Cello-oligosaccharides: Intensification and Scale-up of Their Enzymatic Production and Selective Growth Promotion among Probiotic Bacteria. *Journal of Agricultural and Food Chemistry*, 68(32), 8557–8567.

LIST OF PUBLICATION

1. Ismail, N. F., Abang Zaidel, D. N., and Mat Isa, M. N. (2021). Characterization of Thermostable Beta-1,4-Galactanase and Its Application in Hydrolysis of Pectin from Sweet Potato (*Ipomoea Batatas* (L.) Lam) Peels. *Jurnal Teknologi*, 83(5), 57-65. https://doi.org/10.11113/jurnalteknologi.v83.17198 (Indexed by Scopus)