BIOPROCESS STRATEGY FOR THE PRODUCTION OF *LACTOBACILLUS RHAMNOSUS* NRRL B442 WITH HIGH CELL-B-GLUCOSIDASE ACTIVITY

KOK FOOK SENG

UNIVERSITI TEKNOLOGI MALAYSIA

FERMENTATION STRATEGY FOR THE PRODUCTION OF *LACTOBACILLUS RHAMNOSUS* NRRL B442 WITH HIGH CELL-β-GLUCOSIDASE ACTIVITY

KOK FOOK SENG

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

Faculty of Chemical Engineering
Universiti Teknologi Malaysia

JANUARY 2014

Specially dedicated to my beloved family members. May this achievement inspire them.

ACKNOWLEDGMENT

I would like to express my deepest appreciation to my supervisors, PM. Dr. Ida Idayu Muhamad, PM. Dr. Lee Chew Tin and PM. Dr. Firdausi Razali for their guidance, encouragement and advices.

Special thanks go to the laboratory staffs of Bioprocess Engineering Department, who provided technical assistance throughout the research work.

I would also like to acknowledge with much appreciation the Agricultural Research Service (ARS) for providing the bacteria strain of *Lactobacillus rhamnosus* NRRL B442 in this research work.

Last but not least, a sincere appreciation goes to my beloved family members for their endless love and support.

ABSTRACT

This study aims to strategize the fermentation process for the production of probiotic with high cell-β-glucosidase (CBG) activity using *Lactobacillus Rhamnosus* NRRL B442 as model microbe. The fermentations were carried out in a 2L bioreactor under anaerobic condition, and CBG activity was measured using the standard substrate of O-nitrophenyl-β-D-glucopyranoside (O-NPG). The fermentation parameters were investigated using factor-by-factor optimization method. The cell pellet from shake flask culture was used in the determination of optimum testing condition for the measurements of CBG activity. The condition at 46^{o}C and pH 6.5 was found to be optimum for the measurement of CBG activity. The strategy began with the optimization of the fermentation condition for the maximum production of biomass and CBG activity in a batch system. Four fermentation parameters were investigated, and these include pH, temperature, type of nitrogen source, and type of carbon source. The results suggested that the fermentation condition at pH 6 and 40 ^{o}C, using yeast extract and glucose as nitrogen and carbon source, respectively provided maximum biomass yield, high CBG activity, and low production cost. In addition, the glucose repression effect on CBG activity was confirmed in the bacteria strain studied. Under this primary optimized condition in batch system, the growth kinetics study was performed based on Monod equation. The maximum specific growth rate, μ_{max}; saturation constant, K_s; yield of biomass, $Y_{x/s}$, and doubling time, τ_d, were 0.4672 h^{-1}, 1.128 g glucose/l, 0.313g dcm/g glucose, and 1.483 h, respectively. Based on the profile of specific CBG activity conducted under batch condition, three direct factors including specific growth rate, starvation, and yeast extract concentration were investigated using the proposed operating tool of Chemostat. Another strategy was proposed to increase the CBG activity. This includes: 1) to maximize the growth rate, 2) to supply high concentration of yeast extract, and 3) to supply low concentration of glucose. Using this strategy, an improved specific CBG activity of 11.24 UE/mg dcm (6.25 folds increase in activity compared to control) was obtained at maximum specific growth rate of 0.264 h^{-1}, low feeding glucose concentration of 20g/l, and high feeding yeast extract concentration of 60 g/l. The enzymatic kinetics of CBG activity was investigated experimentally and found to match the Michaelis-Menten model. The kinetics properties of maximum specific rate of reaction, V_m, and Michaelis-Menten constant, K_m were 7.86 UE/mg dcm and 0.049 mM of O-NPG, respectively. As conclusion, the current study has successfully strategized the bioprocess for the production of *Lactobacillus rhamnosus* NRRL B442 with high CBG activity.

ABSTRAK

Kajian ini bertujuan untuk merangka strategi proses fermentasi untuk penghasilan probiotik yang mempunyai aktiviti sel-β-glucosidase (CBG) yang tinggi dengan menggunakan *Lactobacillus Rhamnosus* NRRL B442 sebagai bakteria model. Fermentasi dijalankan di dalam bioreaktor 2L di bawah keadaan anaerobik, dan aktiviti CBG diukur menggunakan substrat asas, iaitu O-nitrophenyl-β-D-glucopyranoside (O-NPG). Semua parameter dikaji dengan menggunakan kaedah faktor-ke-faktor. Gumpalan sel dari kultur kelalang secara berputar digunakan dalam penentuan keadaan penilaian optimum untuk pengukuran tahap aktiviti CBG. Keadaan pada 46 ^{o}C dan pH 6.5 didapati adalah keadaan optimum dan ia seterusnya diaplikasikan dalam pengukuran tahap aktiviti CBG. Strategi ini bermula dengan mewujudkan keadaan fermentasi yang optimum untuk tujuan penghasilan biomas dan aktiviti CBG yang maksimum dalam sistem kelompok. Empat parameter yang telah dikaji termasuk pH, suhu, sumber nitrogen dan sumber karbon. Keputusan menunjukkan keadaan fermentasi pada pH 6 dan 40 ^{o}C, dengan menggunakan ekstrak yis dan glukosa sebagai sumber nitrogen dan sumber karbon masing-masing, telah menghasilkan hasil biomas yang maksimum, aktiviti CBG yang tinggi, dan kos penghasilan yang rendah. Selain itu, kesan penindasan glukosa terhadap aktiviti CBG telah dipastikan dalam jenis bakteria yang digunakan. Dengan keadaan optimum primer dalam sistem kelompok, kajian kinetik pertumbuhan sel dilakukan berdasarkan persamaan Monod. Kadar pertumbuhan maksimum spesifik, μ_{max}, ketepuan malar, K_s, hasil biomas, $Y_{x/s}$, dan masa penggandaan, τ_d, adalah masing-masing, 0.4672 h^{-1}, 1.128 g glukosa g/l, 0.313 g dcm/g glukosa, dan 1.483 h. Berdasarkan profil aktiviti CBG spesifik yang dijalankan di bawah keadaan kelompok, tiga faktor termasuk kadar pertumbuhan sel spesifik, kelaparan, and kepekatan ekstrak yis dikaji dengan menggunakan Chemostat yang dicadangkan. Kajian ini mencadangkan satu strategi untuk meningkatkan aktiviti CBG iaitu: 1) memaksimakan kadar pertumbuhan sel, 2) penggunaan kepekatan glukosa yang rendah (kebuluran), dan 3)penggunaan kepekatan yis ekstrak yang tinggi. Dengan penggunaan strategi tersebut, peningkatan aktiviti CBG spesifik kepada 11.24 UE/g dcm (6.25 gandaan lebih tinggi dalam aktiviti berbanding dengan 'kawalan') telah diperolehi pada kadar pertumbuhan sel spesifik maksimum iaitu 0.264 h^{-1}, penggunaan kepekatan glukosa yang rendah iaitu 20 g/l, dan penggunaan kepekatan ekstrak yis yang tinggi iaitu 60 g/l. Kinetik aktiviti CBG telah diselidik melalui eksperimen dan menunjukkan ia selaras dengan model Michaelis-Menten. Kajian menunjukan kadar spesifik tindakbalas yang maksimum, V_m, and kemalaran Michaelis-Menten, K_m adalah masing-masing, 7.86 UE/mg dcm dan 0.049 mM O-NPG. Kajian ini telah berjaya merangka strategi bioproses untuk penghasilan *Lactobacillus rhamnosus* NRRL B442 yang mempunyai aktiviti CBG yang tinggi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

B.	-	*Bifidobacterium*
Br^-	-	Bromide
BSβG	-	Broad-specific β-glucosidase
Ca^{+2}	-	Calcium ion
CBG	-	Cell-β-glucosidase
$C_2H_3NaO_2$	-	Sodium Acetate
Cl^-	-	Chloride
ClO_4^-	-	Perchlorate
Cu^{+2}	-	Copper (II)
Cs^+	-	Cesium ion
dcm	-	Dry cell mass
DEBG	-	Direct enzyme β-glucosidase
F	-	Flavonoid aglycone
F^-	-	Fluoride
Fe^{+2}	-	iron (II)
Fg	-	Flavonoid glucuronide
FG	-	Flavonoid glucoside
Fs	-	Flavonoid sulphate
HCl	-	Hydrochloric acid
HPLC	-	High-Performance Liquid Chromatography
I^-	-	Iodide
K^+	-	Potassium ion
KH_2PO_4	-	Potassium phosphate
L.	-	*Lactobacillus*
Li^+	-	Lithiu ion
LPH	-	Lactase phloridzin hydrolase

$MgSO_4$	-	Magnesium sulphate
$MnSO_4$	-	Manganese sulphase
MRP	-	Multidrug resistance-associated proteins
Na^+	-	Sodium ion
Na_2HPO_4	-	Disodium hydrogen phosphate
NaOH	-	Sodium hydroxide
NH_4^+	-	Ammonium ion
NO_3^-	-	Nitrate
OAc^-	-	Acetate
O-NPG	-	O-nitrophenly-β-Dglucopyranoside
p-NPG	-	*p*- nitrophenly-β-Dglucopyranoside
Rb^+	-	Rubidium ion
S.	-	*Streptococus*
SD	-	Standard deviation
SO_4^{2-}	-	Sulfate
SGLT	-	Sodium-dependent glucose transporter
SULT	-	Sulfotransferase
UE	-	Unit enzyme
UGT	-	UDP-glucuronosyltransferase
Zn^{+2}	-	Zinc ion

LIST OF SYMBOLS

D	-	Dilution rate
E	-	Enzyme
k_d	-	Death rate
K_m	-	Saturation constant
K_s	-	Michaelis-Menten constant
X	-	Biomass concentration
X_o	-	Initial biomass concentration
S	-	Substrate
T	-	Time
V	-	Rate of reaction
V_m	-	Maximum rate of reaction
μ	-	Specific growth rate
μ_m	-	Maximum specific growth rate

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background and Definition

β-Glucosidase is an enzyme that catalyses the hydrolysis of glycosidic bond linking β-glucose and glucose-substituted substrates. The target substrate of this enzyme is also known as β-glucan. Figure 1.1 illustrates the enzymatic activity of β-glucosidase upon one of its target molecules of quercetin-β-glucoside.

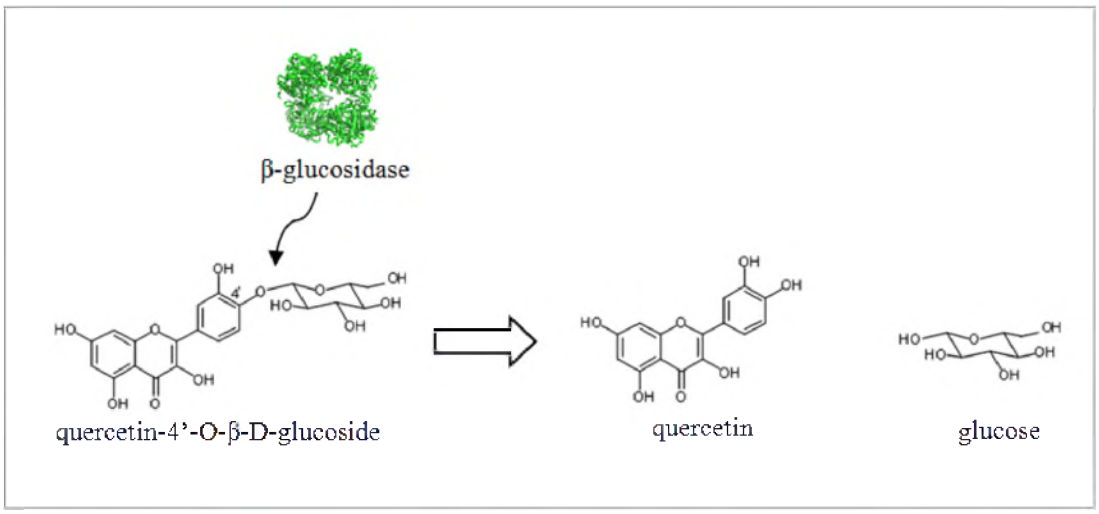

Figure 1.1 Enzymatic activity of β-glucosidase

Enzyme β-glucosidase is widely found in the microorganisms such as fungi (Kaur *et al.*, 2007), yeast (Villena *et al.*, 2006) and lactic acid bacteria (Marazza *et al.*, 2009). For the microorganisms, this enzyme is an essential tool for hydrolysis of β-glucans to produce simple sugar of glucose for growth.

The activity of enzyme β-glucosidase to catalyse the hydrolysis of various β-glucans has brought to the great concern of researchers due to its wide applications in different industries. In food industry, this enzyme is used to improve the aroma properties in wine (Gallifuoco *et al.*, 1998) and tea beverage (Su *et al.*, 2009), also helps to reduce the citrus bitterness of juices (Villena *et al.*, 2006). In another way, the enzyme β-glucosidase activity posed by the lactic acid bacteria is used to produce bioactive isoflavone aglycone in soymilk (Tsangalis *et al.*, 2004; Marazza *et al.*, 2009). Besides, this enzyme also plays important role in hydrolysis of cellulose waste to release glucose for ethanol production (Bhat and Bhat, 1997).

The wide application of the enzyme has brought to the increasing interest of many. However, the expensive process cost for enzyme extraction and purification is not economically susceptible in large scale industrial production especially for those low end products. As such, another alternative is suggested in which the probiotic possessing β-glucosidase activity is used as the catalyst instead of using the expensive purified enzyme. This concept has been successfully proven by Marazza and co researchers (2009) who used the lactic acid bacteria as starter culture in soymilk fermentation. The isoflavone glucosides were hydrolysed into bioactive isoflavone aglycone by the enzyme β-glucosidase released by the bacteria.

In relation to this concept, the present study defined the ability of cells to break the β-glucosidic bond through the expression of enzyme β-glucosidase as cell-β-glucosidase (CBG) activity. The mechanism of the CBG activity upon one of its target molecule of quercetin-4'-β-D-glucoside is illustrated in Figure 1.2.

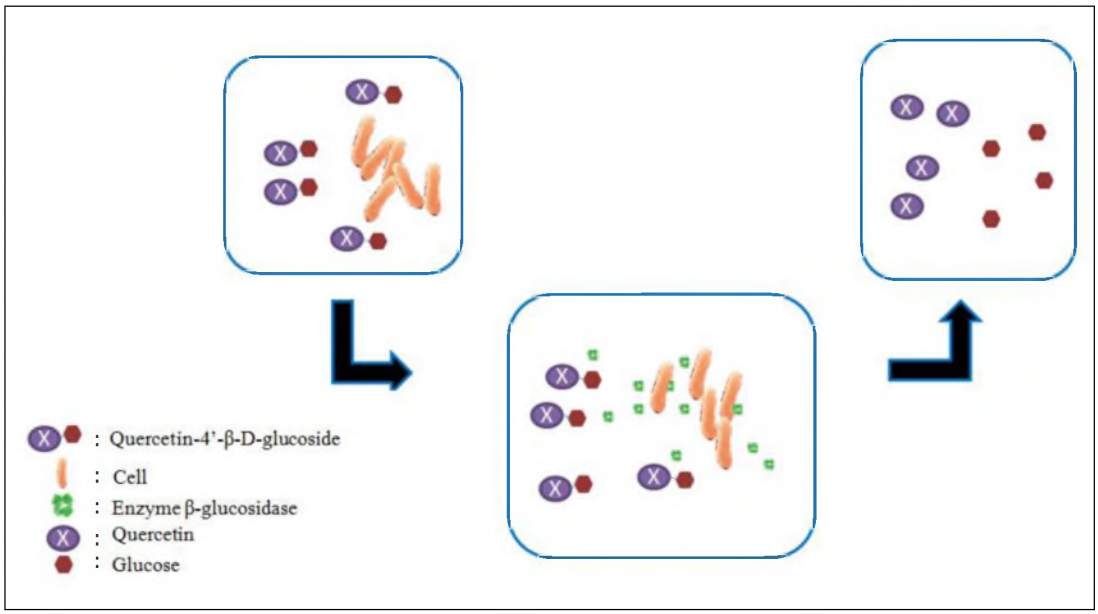

Figure 1.2 The mechanism of cell β-glucosidase activity.

On the other hand, the CBG activity of probiotic has more applications other than that of catalyst. Being the probiotic in human intestinal tract, its CBG activity may function to improve the anti-oxidation quality of the oral consumption of quercetin glucosides from onion (Murakami *et al.*, 2008), and also helps to facilitate their intestinal absorption (Murota and Terao, 2003). For these reasons, the CBG activity becomes one of the important functions of probiotic in human intestinal tract. The probiotic with high CBG activity may become one of the keys to increase its market value.

For these novelties, the production of probiotic with high CBG activity is remarkably important in many aspects. Based on the previous reports, enzyme synthesis of β-glucosidase is significantly affected by the fermentation condition. Generally, the studies achieved an agreement that the enzyme synthesis of β-glucosidase is growth-dependent. Besides, the presence of certain nitrogen and carbon sources may probably lead to the induction or suppression effect on the enzyme synthesis of β-glucosidase (Mahajan *et al.*, 2010; Joo *et al.*, 2010; Villena *et al.*, 2007).

The objective of the present study is to strategize the fermentation system for the production of high CBG activity probiotic. Based on the literature, the high potential probiotic of *Lactobacillus rhamnosus* is used as the study model. In this study, several fermentation parameters were investigated and optimized. This study proposed a bioprocess strategy to overcome the limitations and to control the key factors identified for the improvement of CBG activity. By the end of this study, a hypothesis is made where the CBG activity of the probiotic is improved to a significant extent by the bioprocess strategy proposed.

1.2 Objective

To strategize the fermentation process for the production of probiotic with high cell-β-glucosidase activity using *Lactobacillus rhamnosus* NRRL B442 as model microbe.

1.3 Scopes

i. To determine the optimal testing condition (pH and temperature) for the CBG activity of *Lactobacillus rhamnosus* NRRL B442.

ii. To investigate the effects of fermentation pH, temperature, nitrogen source, and carbon source on the cell growth and CBG activity of *Lactobacillus rhamnosus* NRRL B442 in batch system.

iii. To determine the growth kinetics properties of *Lactobacillus rhamnosus* NRRL B442 in batch system based on Monod equation.

iv. To strategize fermentation process for the production of high CBG activity of *Lactobacillus rhamnosus* NRRL B442 using chemostat strategy.

v. To determine the enzyme kinetics of CBG activity of *Lactobacillus rhamnosus* NRRL B442 produced under the strategized chemostat condition.

1.4 Novelties of Study

I. In this study, *Lactobacillus rhamnosus* NRRL B442 is used as the model microbe. Up to now, the related study on this bacteria strain is relatively rare. This study is reporting the fermentation data that describes the response of this bacteria strain to the environment change. These include: the determination of optimal growing environment, growth kinetics and limitations of the fermentation process.

II. This study proposed a fermentation strategy to improve the CBG activity of the bacteria strain up to a significant extent. This strategy also provided a basic guideline in selecting the operating system and the fermentation condition.

1.5 Thesis Outline

Generally, present study reported the development of bioprocess strategy to improve the CBG activity of *Lactobacillus rhamnosus* NRRL B442. This dissertation consists of seven chapters. Chapter 1 introduces the research background and objectives of study. Chapter 2 reviews the related fundamental

knowledge and reported case studies. The literature review includes the novelty and application of CBG activity of probiotic, fermentation technology for growing of lactic acid bacteria and also the potential key factors for development of the bioprocess strategy to improve the CBG activity. Chapter 3 describes the research methodology with the detail of working protocols and experimental designs. Chapter 4 investigates the effect of reaction pH and temperature on the CBG activity. The optimal values identified are applied in the measurements of CBG activity in the following experimental phase. Chapter 5 investigates the effect of fermentation parameters (pH, temperature, nitrogen source, and carbon source) on the cell growth and CBG activity. This investigation identified the potential key factors, and thus suggested the switch of the operational tool to enable better control of the key factors to improve the CBG activity. Prior to the process strategy, the growth kinetic properties in batch system based on the Monod equation are first determined. Chapter 6 validates the process strategy as suggested in Chapter 5 to improve the CBG activity using a different operational tool of chemostat. Besides, the ability of cell to retain its high CBG activity after the storage at low temperature was discussed. This chapter also describes the kinetic properties of the CBG activity using Michaelis-Menten kinetic model. Finally, Chapter 7 summarizes the overall findings and also suggestions for the future work.

REFERENCES

Adamberg, K., Kaska, S., Laht, T. and Paalme, T. (2003). The Effect Of Temperature And Ph On The Growth Of Lactic Acid Bacteria: A Ph-Auxostat Study. *International Journal of Food Microbiology*. 85, 171–183.

Aguirre-Ezkauriatza, E. J., Aguilar-Yáñez, J. M., Ramírez-Medrano, A., and Alvarez, M. M. (2010). Production Of Probiotic Biomass (*Lactobacillus Casei*) In Goat Milk Whey: Comparison Of Batch, Continuous And Fed-Batch Cultures. *Bioresource Technology*. 101, 2837–2844.

Apostolidis, E. Kwon, Y. and Shetty, K. (2007), Inhibitory Potential Of Herb, Fruit And Fungal-Enriched Chese Against Key Enzymes Linked To Type 2 Diabetes And Hypertension. *Innovative Food Science and Engineering Technologies*. 8, 46-54.

Bajpai-Dikshit, J., Suresh, A. K. and Venkatesh, K. (2003). An Optimal Model for Representing the Kinetics of Growth and Product Formation by *Lactobacillus rhamnosus* on Multiple Substrates, *Journal of Bioscience and* Bioengineering. 96 (5), 481–486.

Bhat, M. K. and Bhat, S. (1997). Cellulose Degrading Enzymes And Their Potential Industrial Application. *Biotechnology Advances*. 15(3), 583-620.

Bokkenheuser, V. D., Shackleton, C. H. L. and Winter, J. (1987). Hydrolysis Of Dietary Flavonoid Glycosides By Strains Of Intestinal Bacteroides From Humans. *Biochemical Journal*. 248 (3), 953-956.

Bowers, E.M., Ragland, L.O. and Byers, L.D. (2007). Salt Effects On B-Glucosidase: pH-Profile Narrowing. *Biochimicaet Biophysica Acta*. 1774, 1500–1507.

Busairi, A.M. and Mat, H. (2002). *Lactic Acid Fermentation Of Pineapple Waste By Lactobacillus Delbrueckii*. Doctor Philosophy, Universiti Teknologi Malaysia, Skudai.

Cheng, P., Mueller, R. E., Jaeger, S., Bajpai, R., and Iannotti, E. L. (1991). Lactic Acid Production From Enzyme-Thinned Corn Starch Using *Lactobacillus Amylovorus*. *Journal Of Industrial Microbiology*. 7, 27-34.

Chun, J., Kim, J. S. and Kim, J. H. (2008). Enrichment Of Isoflavone Aglycones In Soymilk By Fermentation With Single And Mixed Cultures Of *Streptococcus Infantarius*12 And *Weissella* Sp.4. *Food Chemistry*. 109, 278-284.

Chen, M., Qin, Y., Liu, Z., Liu, K., Wang, F., and Qu, Y. (2010). Isolation And Characterization Of B-Glucosidase From Penicillium Decumbens And Improving Hydrolysis Of Corncob Residue By Using It As Cellulase Supplementation. *Enzyme And Microbial Technology*. 46, 444–449.

Chien, H. L., Huang, H. Y. and Chou, C. C. (2006). Transformation Of Isoflavone Phytoestrogens During The Fermentation Of Soymilk With Lactic Acid Bacteria And Bifidobacteria. *Food Microbiology*. 23, 772-778.

Cort, D. S., Kumar, S. H. M. C., and Vrachtert, H. (1994). Localization And Characterization Of A-Glucosidase Activity In *Lactobacillus Brevis*. *Applied And Environmental Microbiology*. 46-63.

Corzo-Martineza, M., Corzoa, N., and Villamiel, M.A. (2007). Biological Properties Of Onion And Garlic, *Food science and technology*. 18, 609-625.

Coulon, S., Chemarin, P., Gueguen, Y., Arnaud, A. and Galzy, P. (1998). Purification And Characterization Of An Intracellular Beta-Gucosidase From Lactobacillus Casei ATCC393. *Appl. Biochem. Biotechnol.* 74(2), 105-114.

Cuzens, J. And Miller, J.R. (1997). Acid Hydrolysis Of Bagasse For Ethanol Production. *Renewable Energy*. 10 (2/3), 285-290.

Gallifuoco, A., D'Ercole, L., Alfani, F., Cantarella, M., Spagna, G., Pifferi, P.G. (1998). On The Use Of Chitosan-Immonilized B-Glucosidase In Wine-Making: Kinetics And Enzyme Inhibition. *Process Biochemistry*. 2, 163-168.

Gerez, C. L., Torino, M. I., Rollán and G. and Valdez G. G. D (2009), Prevention Of Bread Mould Spoilage By Using Lactic Acid Bacteria With Antifungal Properties. *Food Control*. 20, 144–148.

Goksungur, Y., and Guvenc, U. (1997). Batch And Continuous Production Of Lactic Acid From Beet Molasses By *Lactobacillus Debrueckii* IFO3202. *J. Chem. Biotechnology*. 69, 399-404.

Gonzalez-Vara, A. Y. R., Pinelli, D., Rossi, M., Fajner, D., Magelli, F., and Matteuzzi, M. (1996). Production Of L(+) And D(-) Lactic Acid Isomers By

Lactobacihs Casei Subsp. *Casei* DSM 20011 And *Lactobacillus Coryniformis* Subsp. *Torquens* DSM 20004 In Continuous Fermentation. *Journal of Fermentation and Bioengineering*. 81(6), 548-552.

Gullon, B., Yanez, R., Alonso, J.L. and Parajo, J.C. (2008). L- Lactic acid Production From Apple Pomace By Sequential Hydrolysis And Fermentation, *Bioresource Technology*. 99, 308-319.

Grabovac, V. and Bernkop-Schnurch, A. (2006). Improvement Of The Intestinal Membrane Permeability Of Low Molecular Weight Heparin By Complexation With Stem Bermelain, *International Journal of Pharmaceutic*. 326,153-159.

Guggi, D. and Bernkop-Schnurch, A. (2005), Improved paracellular uptake by the combination of different types of permeation enhancers, *International Journal of Pharmaceutic*, 288: 141-150.

Ha, M.Y., Kim, S.W., Lee, Y.W., Kim, M.J., and Kim, S.J. (2003). Kinetics Analysis of Growth and Lactic Acid Production in pH-Controlled Batch Cultures of *Lactobacillus casei* KH- 1 Using Yeast extract/Corn Steep Liquor/Glucose Medium, *Journal of Bioscience and Bioengineering*,. 96(2), 134- 140.

Hang, Y. and Chen, H. (2008), Characterization Of B-Glucosidase From Corn Stover And Its Application In Simultaneous Saccharification And Fermentation. *Bioresource Technology*. 99, 6081–6087.

Hernandez, L. F., Espinosa, J. C., Fernandez-Gonzalez, M. and Briones, A. (2003). β-Glucosidase Activity In A *Saccharomyces Cerevisiae* Wine Strain. *International Journal of Food Microbiology*, 80, 171–176.

Hjorleifsdottir, S., Seevaratnam, S., Holst, O. and Mattiasson, B. (1990), Effects of Complete Cell Recycling on Product Formation by *Lactobacillus casei* ssp. *rhamnosus* in Continuous Cultures. *Current Microbiology*. 20, 287-292.

Hujanen, M. and Linko, Y.Y (1996). Effect Of Temperature And Various Nitrogen Sources On L (+)- Lactic Acid Production By *Lactobacillus Casei*. *Appl. Microbiol. Biotechnology*. 45, 307-313.

Jager, S., Brumbauer, A., Feher, E., Reczey, K., and Kiss, L. (2001). Production And Characterization Of B-Glucosidase From Different *Aspergillus* Strains, *World Journal of Microbiology and Biotechnology*. 17, 455-461.

Joo, A., Jeya, M., Lee, K., Lee, K., Moon, H., Kim, Y. and Lee, J. (2010). Production And Characterization Of B-1,4-Glucosidase From A Strain Of *Penicillium Pinophilum*, *Process Biochemistry*. 45, 851–858.

Kask, S., Adamberg, K., Orlowski, A., Vogensen, F.K., Møller, P.L., Ardo, Y., and Paalmea, T. (2003). Physiological Properties Of *Lactobacillus Paracasei*, *L. Danicus* And *L. Curvatus* Strains Isolated From Estonian Semi-Hard Cheese, *Food Research International*. 36, 1037–1046.

Kaur, J., Chadha, B.S., Kumar, B.A., Kaur, G.S. and Saini, H.S. (2007). Purification and Characterization of β-Glucosidase from *Melanocarpus* sp. MTCC 3922. *Electronic Journal of Biotechnology*. 10 (2), 260-270.

Kobayashi, H., Yamakoshi, Y., Hosaka, Y., Yabushita, M. and Fukuoka, A. (2013). Production of sugar alcohols from real biomass by supported platinum catalyst. Catalysis Today. , http://dx.doi.org/10.1016/j.cattod.2013.09.057.

Korish, M. and Salem, M. (2007). A Broad-Specificity B-Glucosidase From A Wild Type Of Yeast Isolate And Its Potential Use In Food Industry. *Alex. J. Fd. Sci. and Tecnol.*. 4 (1), 63-72.

Konda,T., Yano, H. And Yoshinaga, F. (1997), Effect Of Agitator Configuration On Bacterial Cellolose Productivity In Aerated And Agitated Culture, *Journal of Fermentation and Bioengineering*. 83(4), 371-376.

Kristo, E., Biliaderis, C.G. and Tzanetakis, N. (2003), Modelling Of Acidification Process And Rheological Properties Of Milk Fermented With Yogurt Starter Culture Using Response Surface Methodology. *Food Chemistry*. 83, 437-446.

Lavarack, B.P., Griffin, G.J. and Rodman, D. (2002). The Acid Hydrolysis Of Sugarcane Bagasse Hemicelluloses To Produce Xylose, Arabinose, Glucose And Other Products. *Biomass and Bioenergy*. 23, 367-380.

Lecas, M., Gunata, Z., Sapis, J.C. and Bayonove, C. (1991). Purification And Partial Characterization Of Beta-Glucosidase From Grapes. *Phytochemistry*. 30, 451–454.

Lee, J.H., Kwon, K.S. and Hah, Y.C. (1996). Regulation Of B-Glucosidase Biosynthesis In *Aspergillus Nidulans*. *FEMS Microbiology Letters*. 135, 79-83.

Li, K. B. and Chan, K.Y. (1983), Production And Properties Of Alpha-Glucosidase From Lactobacillus Acidophilus. *Appl. Environ. Microbiol.*. 46(6), 1380-1387.

Li, Z., Han, L., Ji, Y., Wang, X. and Tan, T. (2010). Fermentative Production Of L-Lactic Acid From Hydrolysate Of Wheat Bran By *Lactobacillus Rhamnosus*, *Biochemical Engineering Journal*. 49, 138–142.

Liew, S. L., Ariff, A. B., Raha, A. R. and Ho, Y.W. (2005). Optimization Of Medium Composition For The Production Of A Probiotic Microorganism, *Lactobacillus Rhamnosus*, Using Response Surface Methodology. *International Journal of Food Microbiology*. 102, 137-142.

Lombard, K., Peffley, E., Geoffriau, E., Thompson, L. and Herring, A. (2005). Quercetin In Onion (*Allium Cepa* L.) After Heat-Treatment Simulating Home Preparation. *Journal of Food Composition and Analysis*. 18, 571-581.

Lotito S.B. and Frei, B. (2006), Consumption Of Flavonoid-Rich Foods And Increased Plasma Antioxidant Capacity In Humans: Cause, Consequence, Or Epiphenomenon. *Free Radical Biology & Medicine*. 41, 1727–1746.

Lund, B., Nordahl, B. and Ahring, B. (1992). Production Of Lactic Acid From Whey Using Hydrolysed Whey Protein As Nitrogen Source. *Biotechology letters*. 14, 851-856.

Ly, T.N., Hazama, C., Shimoyamada, M., Ando, H., Kato, K. and Yamauchi, R. (2005), Antioxidative Compounds From The Outer Scales Of Onion, *Journal of Agricultural and Food Chemistry*. 53 (21), 8183–8189.

Madigan, M. T., Martinko, J. M. and Parker (2003), Brock Biology of Microorganism. 10th. Ed. Upper Saddle River, N.J.: Prentice Hall.

Magalhaes, L.M, Segundo, M.A., Reis, S. And Lima, J. L. F. C. (2008), Methodological Aspects About In Vitro Evaluation Of Antioxidant Properties, *Analytica Chimica Acta*. 613, 1-19.

Mahajan, P. M., Desai, K. M. and Lele, S.S. (2010). Production Of Cell Membrane-bound α- and β-glucosidase By *Lactobacillus Acidophilus*. *Food Bioprocess Technol*. DOI 10.1007/s11947-010-0417-2.

Maldonado, A., Ruiz-Barba, J. L. and Jimenez-Diaz, R. (2003), Production Of Plantaricin NC8 By Lactobacillus Plantarum NC8 Is Induced In The Presence Of Different Types Of Gram-Positive Bacteria. *Arch Microbiol.*. 181, 8-16.

Marazza, J. A., Garro, M. S. and Giori, G.S.D. (2009). Aglycone Production By *Lactobacillus Rhamnosus* CRL981 During Soymilk Fermentation. *Food Microbiology*. 26, 333-339.

Mataragasa, M., Metaxopoulosa, J., Galiotoub, M. and Drosinosa, E. H. (2003). Influence Of Ph And Temperature On Growth And Bacteriocin Production By *Leuconostocmesenteroides* L124 And *Lactobacillus Curvatus* L442, *Meat Science*. 64, 265–276.

Mel, M., Karim, M.I.A, Salleh, M.R.M. and Abdullah, R. (2007). Determination Of Critical Point Of Po_2 Level In The Production Of Lactic Acid By *Lactobacillus Rhamnosus*. *Journal of Applied Science*. 7 (4), 523-526.

Mombelli, B. and Gismondo, M.R. (2000). The Use Of Probiotics In Medical Practice. *International Journal of Antimicrobial Agents*. 16, 531–536

Murakami, A., Ashida, H. And Terao, J. (2008), Multitargeted Cancer Prevention By Quercetin. *Cancer Letters*. 269, 315-325.

Murota, K. And Terao. J (2003), Antioxidative Flavonoid Quercetin: Implication Of Its Intestinal Absorption And Metabolism, *Archives of Biochemistry and Biophysic*. 417, 12-17.

Murray, P., Aro, N., Collins,C., Grassick, A., Penttilä, M., Saloheimo, M., and Tuohy, M. (2004). Expression In *Trichoderma Reesei* And Characterisation Of A Thermostable Family 3 B-Glucosidase From The Moderately Thermophilic Fungus *Talaromyces Emersonii, Protein Expression and Purification* . 38, 248–257.

Nancib, A. Nancib, N., Meziane-Cherif, D., Boubendir, A., Fick, M. and Boudrant, J. (2005). Joint Effect Of Nitrogen Sources And B Vitamin Supplementation Of Date Juice On Lactic Acid Production By *Lactobacillus Casei Subsp.Rhamnosus*. *Bioresource Technology*. 96, 63-67.

Nuutila, A.M., Kammiovirta, K. And Oksman-Caldentey, K. M. (2012).Comparison Of Methods For The Hydrolysis Of Flavonoids And Phenolic Acids From Onion And Spinach For HPLC Analysis. *Food Chemistry*. 76, 519-525.

Perez-Martin, F., Sesena, S., Izquierdo, P.M., Martin, R., Palop, M.L. (2012), Screening For Glycosidase Activities Of Lactic Acid Bacteria As A Biotechnological Tool In Oenology, *World J. Microbiol Biotechnol.*. 28, 1423–1432.

Pinelli, D., Gonzalez-Vara, A., Matteuzzi, D., and Magelli, F. (1997). Assessment Of Kinetics Models For The Production Of L- And D- Lactic Acid Isomers By *Lactobacillus Casei* DSM 20011 And *Lactobacillus Cornıformis* DSM 20004

In Continuous Fermentation. *Journal of Fermentation and Bioengineering*. 2, 209-212.

Pitson, S.M., Seviour, R.J. and Mcdougall, B.M. (1999). Induction And Carbon Source Control Of Extracellular B-Glucosidase Production In *Acremonium* Persicinum. *Mycol. Res*.. 103(2), 161-167.

Pontoh, J. and Low, N. H. (2002). Purification And Characterization Of B-Glucosidase From Honey Bees (*Apismellifera*). *Insect Biochemistry and Molecular Biol*ogy. 32, 679–690.

Prachyakij, P., Charernjiratrakul, W. and Kantachote., D. (2008). Improvement In The Quality Of A Seaweed Beverage Using An Antiyeast Starter Of *Lactobacillus Plantarum* DW3 And Partial Sterilization. *World J. Mirobiol Biotechnol*. 24, 1713-1720.

Price, K. R., Bacon, J. R. and Rohdes, J. C. (1997). Effect Of Storage And Domestic Processing On The Contents And Composition Of Flavonol Glucosides In Onion (*Allium Capa L.*), *Journal of Agricultural and Food Chemistry*. 45: 938-942.

Price, K.R. and Rohdes, J.C. (1997), Analysis Of The Major Flavonol Glycosides Present In Four Varieties Of Onion (*Allium Cepa*) And Changes In Composition Resulting From Autolysis. *J Sci Food Agric*.. 74, 331-339.

Pyo, Y.H., Lee, T.C. and Lee, Y.C. (2005). Enrichment Of Bioactive Isoflvones In Soymilk Fermented With B-Glucosidase-Producing Lactic Acid Bacteria. *Food Research International*. 38, 551-559.

Rao, M.S., Pintado, J., Stevens, W.F. and Guyot, J.P. (2004). Kinetics Growth Parameters Of Different Amylolytic And Non-Amylolytic *Lactobacillus* Strains Under Various Salt And pH Conditions. *Bioresource Technolog*. 94, 331–337.

Riou, C., Salmon, J., Vallier, M., Gunata, Z., and Barre, P. (1998). Purification, Characterization, And Substrate Specificity Of A Novel Highly Glucose-Tolerant B-Glucosidase From *Aspergillus Oryzae. Applied and Environmental Microbiology*. 64, 3607-3614.

Roberts, J. S. and Kidd, D. R. (2005), Lactic Acid Fermentation Of Onions. *Lebensm. Wiss. U. Technol*. 38,185-190.

Rogosa, M., Franklin, J. G. and Perry, K. D. (1961). Correlation Of The Vitamin Requirements With Cultural And Biochemical Characters Of *Lactobacillus* spp, *J. Gen. Microbiol.*. 25,473-482.

Roldan-Marin, E., Sanchez-Moreno, C., Lloria, R., Ancos, B. D. and Cano, P. M. (2009), Onion High-Pressure Processing: Flovonol Content And Antioxidant Activity. *Food science and Technology*. 42, 835-841.

Sachin, R. K., Sudarshan, S. P., Kulbhushan, B. B., Jayant, M. K., Digambar, V. G. (2006). Strain Improvement Of *Lactobacillus Delbrueckii* NCIM 2365 For Lactic Acid Production. *Process Biochemistry*. 41, 120-126.

Schepers, A. W., Thibault, J. and Lacroixa, C. (2002a). *Lactobacillus Helveticus* Growth And Lactic Acid Production During Ph-Controlled Batch Cultures In Whey Permeate/Yeast Extract Medium. Part I. Multiple Factor Kinetic Analysis. *Enzyme and Microbial Technology*. 30, 176-186.

Schepers, A.W., Thibault, J. and Lacroixa, C. (2002b). *Lactobacillus Helveticus* Growth And Lactic Acid Production During Ph-Controlled Batch Cultures In Whey Permeate/Yeast Extract Medium. Part II:Kinetic Modelling And Model Validation. *Enzyme and Microbial Technology*. 30, 187–194.

Sellappan, S. and Akoh, C. C. (2002), Flavonoids And Antioxidant Capacity Of Georgia-Grown Vidalia Onions. *J. Agric. Food Chem.*. 50 (19), 5338-5342.

Sestelo, A.B.F., Poza, M. And Villa, T. G. (2004). β-Glucosidase Activity In A Lactobacillus Plantarum Wine Strain, *World J. Microbiol. Biotechnol.* 20, 663-637.

Sharififar, F., Dehghn-Nudeh, G. and Mirtajaldini, M. (2008), Major Flavonoids With Antioxidants Activity From *Teucrium Polium*. *Food Chemistry*.112, 885-888.

Shuler, M.L. and Kargi (2002), *Bioprocess Engineering Basic Concepts*, 2th Ed, Prentice Hall, Upper Saddle River NJ.

Silva, E. L. D., Piskula, M. K., Yammoto, N., Moon, J. H. and Terao, J. (1998), Quercetin metabolite inhibit copper ion-induced lipid peroxidation in rat plasma, *FEBS letters*. 430: 405-408.

Skory, D., Freer, S.N., and Bothast, R.J. (1996). Properties Of An Intracellular B-Glucosidase Purified From The Cellobiose-Fermenting Yeast *Candida Wickerhamii. Applied Microbiology And Biotechnology*. 46, 353-359.

Su, E., Xia, T., Gao, L., Dai, Q. and Zhang Z. (2009). Immobilization Of B-Glucosidase And Its Aroma Increasing Effect On Tea Beverage, *Food and Bioproducts Processing*, doi:10.1016/j.fbp.2009.04.001.

Sun-Waterhouse, D., Smith, B. G., O'Connor, C. J., & Melton, L. D. (2008). Effect Of Raw And Cooked Onion Dietary Fibre On The Antioxidant Activity Of Ascorbic Acid And Quercetin. *Food Chemistry*. 111, 580-585.

Takafuji, S., Iwasaki, T., Sasaki, M. and Tan, P.S.T. (1995). Proteolytic Enzymes Of Lactic Acid Bacteria. *Developments in Food Science*. 37, 753-767

Tari, C., Ustok, F. I. and Harsa, S. (2009). Optimization Of The Associative Growth Of Novel Yoghurt Cultures In The Production Of Biomass, B-Galactosidase And Lactic Acid Using Response Surface Methodology. *International Dairy Journal*. 19, 236-243.

Tsangalis D., Ashton, J. F., Stojanovska, L., Wilcox, G. and Shah, N.P. (2004). Development Of An Isoflavone Aglycone-Enriched Soymilk Using Germ, Soy Protein Isolate And Bifidobacteria. *Food Research International*. 37, 301-312.

Valik, L., Medvedova, A. and Liptakova, D. (2008). Characterization Of The Growth Of *Lactobacillus Rhamnosus* GG In Milk At Suboptimal Temperatures. *Journal of Food and Nutrition Research*. 47 (2), 60-67.

Villena, M.A., Iranzo, J.F.U, Gundllapalli, S.B., Otero b, R.R.C. and Perez, A.I.B. (2006). Characterization of an Exocellular β-Glucosidase from *Debaryomyces Pseudopolymorphus*. *Enzyme and Microbial Technology*. 39, 229–234.

Villena, M.A., Iranzo, J.F.U, and Perez, A.I.B. (2007). β-Glucosidase Activity In Wine Yeasts: Application In Enology. *Enzyme and Microbial Technology*. 40, 420–425.

Wallace, R. G. W. (2008). Flavonoid Concentrates, *United States Patent Application Publication*. US2008/0274519 A1.

Walle, T. (2004). Absorption and Metabolism of Flavonoids. *Free Radical Biology and Medicine*. 36(7), 829-837.

Wei, Q. K., Chen, T. R. and Chen, J. T. (2007). Using Of Lactobacillus And Bifidobacterium To Product The Isoflavone Aglycones In Fermented Soymilk. *International Journal of Food Microbiology*. 117, 120-124.

Ximenes, E. A., Felix, C. R., and Ulhoa, C. J. (1996). Production Of Cellulases By *Aspergillus Fumigatus* And Characterization Of One B-Glucosidase. *Current Microbiology*. 32, 119-123.

Xu, R., Ma, S., Wang, Y., Liu, L. and Li, P. (2010), Screening, Identification And Statistic Optimization Of A Novel Exopolysaccharide Producing *Lactobacillus Paracasei* HCT. *African Journal of Microbiology*. 4(9), 783-795.

Yamamoto, N., Moon, J.H, Tsushida, T., Nagao, A. And Terao, J. (1999). Inhibitory Effect Of Quercetin Metabolites And Their Related Derivatives On Copper Ion-Induced Lipid Peroxidation In Human Low-Density Liprotein. *Archives of Biochemistry and Biophysics*. 372(2), 347-354.

Yan, J., Pan, G., Ding, C. and Quan, G. (2010), Kinetics And Thermodynamic Parameters Of B-Glucosidase Immobilized On Various Colloidal Particles From A Paddy Soil. *Colloids and Surfaces B: Biointerfaces*. 79, 298-303.

Yoo, I. K., Chang, H. N., Lee, E.G., Chang, Y. K. , and Moon, S.H. (1997). Effect Of B Vitamin Supplementation On Lactic Acid Production By *Lactobacillus Casei*. *Journal of Fermentation And Bioenginnering*. 84 (2), 172-175.

Yu, H., Xu, J., Lu, W. and Lin, G. (2007). Identification, Purification And Characterization Of B-Glucosidase From Apple Seed As A Novel Catalyst For Synthesis Of O-Glucosides. *Enzyme and Microbial Technology*. 40, 354-361.

Yu, L., Lei, T., Ren, X., Pei, X. and Feng, Y. (2008). Response Surface Optimization Of Lactic Acid Production Using Corn Steep Liquor As An Alternative Nitrogen Source By *Lactobacillus Rhamnosus* CGMCC 1466. *Biochemical Engineering Journal*. 39, 496-502.

Zheng, L., Lu, H., Yizhi, J., Xiaonan, W. and Tianwei, T. (2010). Fermentative Production Of L-Lactic Acid From Hydrolysate Of Wheat Bran By *Lactobacillus Rhamnosus*. *Biochemical Engineering Journal*. 49, 138-142.