UNIVERSITI PUTRA MALAYSIA

EXTRACTION, PURIFICATION, MICROENCAPSULATION AND CHARACTERIZATION OF LIPASE FROM PUMPKIN (CUCURBITA MOSCHATA DUCHESNE EX POIR.) SEED

MUHAINI BINTI MOHD HUSSIN

FSTM 2016 27

EXTRACTION, PURIFICATION, MICROENCAPSULATION AND CHARACTERIZATION OF LIPASE FROM PUMPKIN (*Cucurbita moschata* DUCHESNE EX POIR.) SEED

By

MUHAINI BINTI MOHD HUSSIN

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

December 2016

COPYRIGHT

DEDICATION

I dedicate this thesis to the love of my life... mak, abah and Hanafi….. thank you for being there even in my darkest time…

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

EXTRACTION, PURIFICATION, MICROENCAPSULATION AND CHARACTERIZATION OF LIPASE FROM PUMPKIN (*Cucurbita moschata* DUCHESNE EX POIR.) SEED

By

MUHAINI BINTI MOHD HUSSIN

December 2016

Chairman : Associate Professor Dr Mehrnoush Amid,PhD
Faculty : Food Science and Technology

Lipase is an enzyme with the presence of hydrolases act on ester bonds of triacylglerols. Most of the enzymes are easily degradable when expose to multistep process and expensive such as conventional purification. Hence it is important to establish and develop simple, low cost and environmental friendly system that could produce lipase to be used in industries such as food, detergent, pharmaceutical, biofuel industries. The pumpkin seed constitutes 30-37% of the whole pumpkin possesses valuable enzyme. Hence, pumpkin seed can be a potential novel source for the valuable and economical natural enzyme such as lipase. In this study, lipase was extracted from pumpkin (*Cucurbita moschata*) seed and the effects of the main factors affecting enzyme extraction namely, temperature, extraction time, pH of buffer, and buffer to sample (B/S) ratio were investigated for the development of the ultrasound-assisted extraction method. Optimum extraction condition was achieved at 5.5:1 (w/w) B/S ratio, 45 mins extractiong time, temperature 80 ºC and pH of buffer 8.0. The yield of the enzyme extracted was 80.1%. Subsequently, the potential pplication of novel aqueous two-phase system (ATPS) composed of Triton X-100 nd xylitol in the purification of lipase from pumpkin seed crude was demonstrated t laboratory scale. In this part of the study, the effect of the main important parameters (such as volume ratio, crude load and pH) on purification of the enzyme was investigated. Optimum condition for purification of lipase from pumpkin seed was obtained After that, optimized extracted sample was purified using aqueous two-phase system composed of 22% (w/w) and 25% (w/w) xylitol at 56.2% of tie line length (TLL) and 25% crude at pH 8.0 in order to obtain the purified enzyme. Based on the results it was demonstrated that the temperature TLL, volume ratio, crude load, and pH of buffer influenced the lipase partitioning. In ATPS, it was found that the molecular of lipase was estimated to be 39.2 kDA. Microencapsulation was performed using freeze-drying found that yield of freeze-dried in the trehalose (2%) and Arabic gum (5%) increased to 97.3% ± 0.3. It was found that during storage encapsulated lipase is stable by 95.2% ±0.1. The immobilized lipase was stable at

80^0C compared to the free enzyme, was around 50^0C. Characterization of the purified enzyme showed that lipase from pumpkin seed is stable in the presence of metal ions, surfactant and oxidizing agents. The lipase was stable 80^0C and pH 8 was found to be its optimum pH. The enzyme showed highest residual lipse activity on calcium chloride ($CaCl_2$) and EDTA. Whereas, in substrate specificity, 4-nitrophenyl palmitate showed highest enzyme activity compared to corn oil, olive oil, soybean oil, and palm oil. It can be concluded that the valuable enzyme with unique characteristics from a rich, natural and cost-effective source could be made available for use in different types of industries such as food, detergents and also in biotechnological applications.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGEKSTRATAN , PENULENAN , PEMIKROKAPSULAN DAN PENCIRIAN ENZIM LIPASE DARI BIJI LABU (*Cucurbita moschata* DUCHESNE EX POIR.) SEED

Oleh

MUHAINI BINTI MOHD HUSSIN

Disember 2016

Pengerusi : Profesor Madya Dr Mehrnoush Amid,PhD
Fakulti : Sains dan Teknologi Makanan

Lipase merupakan enzim yang berhidrolas bertindak atas bon ester. Kebanyakan enzim terurai apabila terdedah kepada proses berperingkat dan mahal seperti penyucian konvensional. Oleh itu kaedah mudah, kos rendah dan mesra alam boleh menghasilkan lipase yang akan digunakan dalam industri seperti makanan, bahan pencuci, farmaseutikal, industri biofuel. Biji labu merupakan 30-37% daripada keseluruhan labu mempunyai enzim berharga. Oleh itu, biji labu boleh menjadi sumber novel yang berpotensi untuk enzim semula jadi yang berharga dan ekonomi seperti lipase. Lipase telah diekstrak daripada biji labu (*Cucurbita moschata*) dan kesan faktor utama yang menjejaskan pengeluaran enzim iaitu, suhu, masa pengekstrakan, pH, dan penampan untuk mencuba nisbah telah disiasat untuk pembangunan kaedah pengekstrakan ultrasound bantuan itu. Keadaan pengeluaran yang optimum dicapai pada 5.5: 1 (b/b) B / nisbah S, masa 45 minit pengekstratan, suhu 80 ºC dan pH penampan 8.0. Hasil enzim yang diekstrak adalah 80.1%. Selepas itu, permohonan potensi novel akueus sistem dua fasa (ATP) terdiri daripada Triton X-100 dan Xylitol dalam penulenan lipase dari mentah biji labu telah ditunjukkan pada skala makmal. Di bahagian ini, kajian, kesan parameter penting utama (seperti suhu, beban mentah dan pH) kepada pembersihan enzim itu disiasat. keadaan optimum untuk penulenan lipase daripada biji labu telah diperolehi Selepas itu, sampel diekstrak dioptimumkan telah disucikan menggunakan akueus sistem dua fasa terdiri daripada 22% (w/w) dan 25% (w/w) xylitol pada 56.2% daripada tali leher panjang talian (TLL) dan 25% mentah pada pH 8.0 untuk mendapatkan enzim yang tulen. Berdasarkan keputusan itu telah menunjukkan bahawa suhu TLL, nisbah jumlah, beban mentah, dan pH dipengaruhi pembahagian lipase. Dalam novel akueus sistem dua fasa, didapati bahawa molekul lipase dianggarkan 39.2 KDA. Pemikrokapsulan dilakukan dengan menggunakan beku-pengeringan mendapati bahawa hasil beku-kering menggunakan trehalose (2%) dan gum Arabic (5%) meningkat kepada 97.3% ± 0.3. Ia telah mendapati bahawa semasa penyimpanan

terkandung lipase adalah stabil dengan 95.2% ± 0.1. Lipase stabil pada 80^0C berbanding enzim percuma, adalah sekitar 50^0C. Pencirian enzim yang tulen menunjukkan bahawa lipase daripada biji labu adalah stabil di hadapan kakisan, surfaktan dan agen pengoksidaan. Lipase itu 80^0C stabil dan pH 8 didapati pH optimum. Enzim ini menunjukkan aktiviti lipse sisa tertinggi kalsium klorida ($CaCl_2$) dan EDTA. Manakala, dalam substrat kekhususan, substrat 4-nitrophenyl palmitate menunjukkan aktiviti enzim tertinggi berbanding dengan minyak jagung, minyak zaitun, minyak kacang soya dan minyak sawit. Dapat disimpulkan bahawa enzim berharga dengan ciri-ciri unik dari sumber yang kaya, semula jadi dan kos efektif boleh disediakan untuk digunakan dalam pelbagai industri seperti makanan, bahan pencuci dan juga dalam aplikasi bioteknologi.

ACKNOWLEDGEMENTS

My first and most heartily gratitude goes to the almighty ALLAH who blesses to all for His divine throughout my life, this master programme and this thesis.

First and foremost I wish to express my utmost gratitude to my main supervisor, Associate Professor Dr. Mehrnoush Amid for her continuous guidance and always made me perform better confidentiality during the course of this research. Her perfectionist touch and guidance at every stage of my journey and her dedicated time to edit my writings, discuss about the project and relentless support academically has enabled the completion of this project. I would also like to thank my supervisory committee, Associate Professor Badlishah Sham Bahrin, Professor Dato' Dr Mohd Yazid Manap for their support during the dissertation. Also, I would like to thank Professor Sadequr Rahman and his PhD student, Gopal Ji Tiwari from Molecular Biology Laboratory, Monash University for allowing me to use their materials and apparatus to run SDS-PAGE.

I acknowledge my deep indebtedness to Eilaf Khalil Suliman who despite of her busy schedule spare time for me and helped me at the time of any difficulty. I wish to extend my sincere appreciation to my friend Fadhilah for sharing invaluable knowledge with me and providing supportive environment throughout my research journey. Specially thanks to Fara Syazana and Farhana Azmira for their valuable support. I can't imagine this whole experience without you guys. I deeply thank to my close friend Wey Zen, Sultan, Hajar for their unforgettable support and motivation which I never forget.

Last but not least, I thank to my parents whom have raised me up and supported me financially and spiritually. Without them, I wouldn't have gone this far.

I certify that a Thesis Examination Committee has met on 23 December 2016 to conduct the final examination of Muhaini binti Mohd Hussin on her thesis entitled "Extraction, Purification, Microencapsulation and Characterization of Lipase from Pumpkin (*Cucurbita moschata* Duchesne ex Poir.) Seed" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Russly bin Abdul Rahman, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Anis Shobirin binti Meor Hussin, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Alfi Khatib, PhD
Associate Professor
International Islamic University Malaysia
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 February 2017

The thesis submitted to the Senate of University Putra Malaysia has been accepted as fulfillment of the requirement for the degree Master of Science. The members of the Supervisory Committee were as follows:

Mehrnoush Amid, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Mohd Yazid Manap, PhD
Profesor
Institut Penyelidikan Produk Halal
Universiti Putra Malaysia
(Member)

Badlishah Sham Bahrin,PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____Date: _____

Name and Matric No.: Muhaini Binti Mohd Hussin (GS40373)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Associate Professor Dr. Mehrnoush Amid

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Mohd Yazid Manap

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Badlishah Sham Bahrin

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

ATPS	Aqueous Two Phase System
BSA	Bovine Serum Albumin
p-NPP	4-nitrophenyl palmitate
EC	Enzyme Commission
EDTA	Ethylenediaminetetraacitic Acid
Mt	Metric ton
MW	Molecular weight
RSM	Response Surface Methodology
SD	Standard Devation
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel electrophoresis
TCA	Trichloroacetic Acid
TLL	Tie Line Length

cm	centimeter
g	gram
M	molarity
mg	milligram
ml	milliliter
mm	millimeter
mM	millimole
µm	micromillimeter
nm	nanometer
kV	kilovolt
U	Unit
v	volume
w	weight

NOMENCLATURE

A_T	Enzyme activity in top phase	U/mL
A_B	Enzyme activity in bottom phase	U/mL
K_e	Partition Coefficient of enzyme	-
K_p	Partition Coefficient of protein	-
P_F	Purification factor of enzyme	-
P_A	Protein concentration of enzyme in top phase	mg/mL
P_B	Protein concentration of enzyme in bottom phase	mg/mL
S	Specific Activity	U/mL
T_A	Total activity of enzyme	U/mL
T_P	Total protein of enzyme	mg
Y	Yield of enzyme	%

CHAPTER 1

INTRODUCTION

1.1. Background of Study

Pumpkin is derived from the genus *Cucurbita* of the family *Cucurbitaceae* and is grown in the tropical and sub tropical regions. Pumpkin is processed into byproducts such as fried, frozen, candied, dried or pickled (Mayor *et al.*, 2007) which have gained attention and popularity not only from the consumer but also from the manufacturer. In Malaysia, *C.moschata* is the most common pumpkin and it is known as *Labu manis* in Malay language. *C.moschata* was selected for extraction and purification of lipase in this study.

Lipase acts as a catalyst during hydrolysis of triacylglycerols which release fatty acids and glycerols. Lipase is important as it involves in a number of reactions such as esterification, interesterification, acidolysis and aminolysis and making it the most versatile biocatalyst (Pandey *et al.*, 2010). Besides that, lipase is characterized with its ability to operate in mild conditions. It also possesses unique specificities that direct the reaction course towards a desired product (Villeneuve, 2003). The lipase market has been growing rapidly and up to this date, its is increasing 8% annually and in the future it is expected to reach 30 billion Euros (Villeneuve, 2003). Therefore, it is very important to establish an alternative especially from different natural source such as plant seed.

The conventional classical extraction methods such as soxhlet extraction and solvent extraction have many disadvantages, one of them is the requirement of several hours of contact times (Albu *et al.*, 2004). Application of ultrasound in extraction is an alternative to overcome this drawback as it is proven to give greater impact in extraction process. The efficiency of extraction is increased by ultrasound due to cavitation (Vilkhu *et al.*, 2008).

Generally, the ultrasound assisted extraction (UAE) of enzymes is followed by conventional purification steps. This two-step process makes the downstream processing consumed up to 50% to 80% of the final cost of the industrial products (de Brito Cardoso *et al.*, 2014). Therefore, an alternative system is used to simplify downstream processes and remove clarification and desalting steps. Based on this description, aqueous two-phase system (ATPS) was used to purify lipase from pumpkin (*C.moschata*) seed.

ATPS has been an attractive technique for purification and recovery of biomolucles namely proteins (Asenjo and Andrews, 2012), enzymes (Barbosa *et al.*, 2011), nucleic acids (Luechau *et al.*, 2009), and other compounds such as alkaloids (Passos

et al., 2013) and antioxidants (Reis *et al.*, 2012). In the past decades, ATPS was formed by combination of two polymers (dextran/polyethylene glycol) (Antov, 2004) or by combination of polymer-salt (PEG/phosphates, sulphates, or chlorides) (Zhao *et al.*, 2011). However, this conventional ATPS requires additional steps such as ultrafiltration, diafiltration and crystallization to eliminate phase-forming elements from the targeted biomolecules (Amid et al., 2015). To improve this conventional ATPS, an economical and environmental friendly method of ATPS with ability to retain enzyme biological and chemical activity is introduced.

Microencapsulation is a technique used for protection, isolation and controlled release (Anjani *et al.*, 2007). Freeze drying is a technique to encapsulate enzyme as it dehydrate all heat-sensitive material (Ezhilarasi *et al.*, 2013). It is important to use and choose stabilizers to coat the enzyme to minimize the risk of deactivation and destabilization of enzymes (Ezhilarasi *et al.*, 2013).

1.2. Statement of Problem

Lipase is easily degraded under extreme pH, temperature and exposure to industrial chemicals which leads to changes in its natural morphology. Another challenge in utilizing enzyme in industries is during its purification and recovery stages where the protocols involved increase the costs of final product by 60-90% and decrease the yield of desired sample (Barbosa *et al.*, 2011) At least four chromatographic steps are required to determine the purity of lipase (Palekar *et al.*, 2000). The current conventional purification processes are basically multistep, with discontinuous stages and above all are time and labor consuming which lead to higher cost and decrease the overall product yield (Aguilar *et al.*, 2008). Another important aspect is the storage of the enzyme. Enzymes are very sensitive and for that there are many factors responsible for its instability and inactivation such as exposure to pH, temperature, binding of metal ions and oxidative stress. These factors could lead to decrease in the lipase activity and stability (Simpson, 2010).

1.3. Significance of Present Study

Brian (2008) reported that approximately 5,500 metric tonnes of pumpkin are generated in food processing industry. The increased demand of pumpkin and its co-products will shoot up the crop's utility and versatility (Aziah and Komathi, 2009). Hence, the pumpkin crop versatility and profitability could be expanded by diversifying its use and utilizing its agricultural by-product waste, seed (Hameed and El Khaiary, 2008).

There is an urgency to develop a relatively fast and cheap process for purification and recovery of the lipase with a high yield and purity to meet the industrial requirement. The important findings that will be investigated is the effects of storage conditions on activity and stability of the pumpkin seed- based enzyme and obtain the best technique to maintain the lipase activity and stability during storage until further use in industry.

1.4 Objective of Study

The aim of this research are:

1. To optimize extraction condition of lipase from pumpkin (*Cucurbita moschata*) seed using Ultrasound Assisted Extraction (UAE)
2. To develop and optimize the purification procedure of lipase from pumpkin (*Cucurbita moschata*) seed using Aqueous Two Phase System (ATPS)
3. To microencapsulate lipase pumpkin (*Cucurbita moschata*) seed using Freeze-Drying Method
4. To characterize enzymatic properties of Lipase from pumpkin (*Cucurbita moschata*) seed.

REFERENCES

Abada, E. A. E. M. (2008). Production and characterization of a mesophilic lipase isolated from *Bacillus stearothermophilus* AB-1. *Pakistan Journal of Biological Sciences*, *11*(8), 1100-1106.

Abdul Hamid, A., Kerk, C. W., Osman, A., & Misran, A. (2009). Effect of boiling and stir frying on total phenolics, carotenoids and radical scavenging activity of pumpkin (*Cucurbita moschato*). *International Food Research Journal*, *16*(1), 45-51.

Akers, N. L., Moore, C. M., & Minteer, S. D. (2005). Development of alcohol/O 2 biofuel cells using salt-extracted tetrabutylammonium bromide/Nafion membranes to immobilize dehydrogenase enzymes.*Electrochimica Acta*, *50*(12), 2521-2525.

Albu, S., Joyce, E., Paniwnyk, L., Lorimer, J. P., & Mason, T. J. (2004). Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for the food and pharmaceutical industry. *Ultrasonics Sonochemistry*, *11*(3), 261-265.

Aguilar, O., Albiter, V., Serrano-Carreón, L., & Rito-Palomares, M. (2006). Direct comparison between ion-exchange chromatography and aqueous two-phase processes for the partial purification of penicillin acylase produced by *E. coli*. *Journal of Chromatography B*, *835*(1), 77-83.

Amid, M., Asmadi, F. A., Hussin, M., Manap, M. Y., Sarker, Z., & Hean, C. G. (2016). A Novel Aqueous Micellar Two Phase System Composed of Surfactant and Mannitol for Purification of Polygalacturonase Enzyme from Durio zibethinus Murry and Recycling Phase Components. *Separation Science and Technology*, (just-accepted).

Amid, M., Murshid, F. S., Manap, M. Y., & Hussin, M. (2015). A novel aqueous micellar two-phase system composed of surfactant and sorbitol for purification of pectinase enzyme from *Psidium guajava* and recycling phase components. *BioMed research international*, *2015*.

Andrews, B. A., Schmidt, A. S., & Asenjo, J. A. (2005). Correlation for the partition behavior of proteins in aqueous two-phase systems: Effect of surface hydrophobicity and charge. *Biotechnology and bioengineering*, *90*(3), 380-390.

Anjani, K., Kailasapathy, K. and Phillips, M., (2007). Microencapsulation of enzymes for potential application in acceleration of cheese ripening. *International Dairy Journal*, 17, 79-86.

Antov, M. G. (2004). Partitioning of pectinase produced by *Polyporus squamosus* in aqueous two-phase system polyethylene glycol 4000/crude dextran at different initial pH values. *Carbohydrate polymers*, *56*(3), 295-300.

Applequist, W. L., Avula, B., Schaneberg, B. T., Wang, Y. H., & Khan, I. A. (2006). Comparative fatty acid content of seeds of four Cucurbita species grown in a common (shared) garden. *Journal of Food Composition and Analysis*, *19*(6), 606-611.

Aravindan, R., Anbumathi, P., & Viruthagiri, T. (2007). Lipase applications in food industry. *Indian Journal of Biotechnology*, *6*(2), 141.

Asenjo, J. A., & Andrews, B. A. (2012). Aqueous two-phase systems for protein separation: phase separation and applications. *Journal of Chromatography A*, *1238*, 1-10.

Asenjo, J. A., & Andrews, B. A. (2011). Aqueous two-phase systems for protein separation: a perspective. *Journal of Chromatography A*, *1218*(49), 8826-8835.

Aziah, A. A., & Komathi, C. A. (2009). Physicochemical and functional properties of peeled and unpeeled pumpkin flour. *Journal of food science*,*74*(7), S328-S333.

Bakás, L. (2000). Influence of encapsulated enzyme on the surface properties of freeze-dried liposomes in trehalose. *Colloids and Surfaces B: Biointerfaces*, *17*(2), 103-109.

Barbosa, H., Slater, N. K., & Marcos, J. C. (2009). Protein quantification in the presence of poly (ethylene glycol) and dextran using the Bradford method. *Analytical biochemistry*, *395*(1), 108-110.

Barbosa, J. M. P., Souza, R. L., Fricks, A. T., Zanin, G. M., Soares, C. M. F., & Lima, Á. S. (2011). Purification of lipase produced by a new source of Bacillus in submerged fermentation using an aqueous two-phase system. *Journal of Chromatography B*, *879*(32), 3853-3858.

Barros, M., Fleuri, L. F., & Macedo, G. A. (2010). Seed lipases: sources, applications and properties-a review. *Brazilian Journal of Chemical Engineering*, *27*(1), 15-29.

Benavides, J., & Rito-Palomares, M. (2008). Practical experiences from the development of aqueous two-phase processes for the recovery of high value biological products. *Journal of Chemical Technology and Biotechnology*,*83*(2), 133-142.

Bhardwaj, K., Raju, A., & Rajasekharan, R. (2001). Identification, purification, and characterization of a thermally stable lipase from rice bran. A new member of the (phospho) lipase family. *Plant Physiology*, *127*(4), 1728-1738.

Bonomo, R. C., Minim, L. A., Coimbra, J. S., Fontan, R. C., da Silva, L. H. M., & Minim, V. P. (2006). Hydrophobic interaction adsorption of whey proteins: effect of temperature and salt concentration and thermodynamic analysis.*Journal of Chromatography B*, *844*(1), 6-14.

Brian, H. (2008). *Utilisation of Squash Waste: A Report Presented in Partial Fulfilment of the Requirements for the Degree of Bachelor of Technology (Honours) in Food Technology at Massey University* (Doctoral dissertation, Massey University, Palmerston North).

Burdurlu, H. S., Koca, N., & Karadeniz, F. (2006). Degradation of vitamin C in citrus juice concentrates during storage. *Journal of Food Engineering*, *74*(2), 211-216.

Caili, F., Haijun, T., Tongyi, C., Yi, L., & Quanhong, L. (2007). Some properties of an acidic protein-bound polysaccharide from the fruit of pumpkin. *Food chemistry*, *100*(3), 944-947.

Caramez, S. M. B., Stefani, M., Medeiros, J. D., Vieira, M. A., Brueske, G. R., De Francisco, A., & Amante, E. R. (2008). Softening of pumpkin seeds (*Cucurbita moschata*) by alkaline maceration. *Journal of food process engineering*, *31*(4), 431-442.

Caro, Y., Villeneuve, P., Pina, M., Reynes, M., & Graille, J. (2000). Lipase activity and fatty acid typoselectivities of plant extracts in hydrolysis and interesterification. *Journal of the American Oil Chemists' Society*, *77*(4), 349-354.

Caruso, F., Trau, D., Möhwald, H., & Renneberg, R. (2000). Enzyme encapsulation in layer-by-layer engineered polymer multilayer capsules.*Langmuir*, *16*(4), 1485-1488.

Cavalcanti-Oliveira, E. D. A., Silva, P. R. D., Ramos, A. P., Aranda, D. A. G., & Freire, D. M. G. (2010). Study of soybean oil hydrolysis catalyzed by Thermomyces lanuginosus lipase and its application to biodiesel production via hydroesterification. *Enzyme research*, *2011*.

Chaiyaso, T., Seesuriyachan, P., Zimmermann, W., & Aran, H. (2012). Purification and characterization of lipase from newly isolated *Burkholderia multivorans* PSU-AH130 and its application for biodiesel production. *Annals of microbiology*, *62*(4), 1615-1624.

Clonis, Y. D. (2006). Affinity chromatography matures as bioinformatic and combinatorial tools develop. *Journal of Chromatography A*, *1101*(1), 1-24.

Cunha, M. T., Aires-Barros, M. R., & Cabral, J. M. S. (2002). Extraction for rapid protein isolation. *Biotechnology and bioprocessing series*, *27*, 321-372.

de Brito Cardoso, G., Mourão, T., Pereira, F. M., Freire, M. G., Fricks, A. T., Soares, C. M. F., & Lima, Á. S. (2013). Aqueous two-phase systems based on acetonitrile and carbohydrates and their application to the extraction of vanillin. *Separation and Purification Technology*, *104*, 106-113.

de Brito Cardoso, G., Souza, I. N., Mourão, T., Freire, M. G., Soares, C. M. F., & Lima, Á. S. (2014). Novel aqueous two-phase systems composed of acetonitrile and polyols: phase diagrams and extractive performance. *Separation and Purification Technology*, *124*, 54-60.

de Escalada Pla, M. F., Ponce, N. M., Stortz, C. A., Gerschenson, L. N., & Rojas, A. M. (2007). Composition and functional properties of enriched fiber products obtained from pumpkin (*Cucurbita moschata Duchesne ex Poiret*).*LWT-Food Science and Technology*, *40*(7), 1176-1185.

Deimel, T. (2007). Important facts about award-winning styrian pumpkin seed oil and your health. http://www.deimel.biz/_biz/info/pumpkinoil.htm. Accessed 15 August 2015

Derde, L. J., Gomand, S. V., Courtin, C. M., & Delcour, J. A. (2012). Characterisation of three starch degrading enzymes: Thermostable β-amylase, maltotetraogenic and maltogenic α-amylases. *Food chemistry*,*135*(2), 713-721.

Desai, K. G. H., & Jin Park, H. (2005). Recent developments in microencapsulation of food ingredients. *Drying technology*, *23*(7), 1361-1394.

Dragovic-Uzelac, V., Pospišil, J., Levaj, B., & Delonga, K. (2005). The study of phenolic profiles of raw apricots and apples and their purees by HPLC for the evaluation of apricot nectars and jams authenticity. *Food chemistry*,*91*(2), 373-383.

Dutra, J. C., Terzi, S. D. C., Bevilaqua, J. V., Damaso, M. C., Couri, S., Langone, M. A., & Senna, L. F. (2008). Lipase production in solid-state fermentation monitoring biomass growth of *Aspergillus niger* using digital image processing. *Applied Biochemistry and Biotechnology*, *147*(1-3), 63-75.

Dutta, D., Dutta, A., Raychaudhuri, U., & Chakraborty, R. (2006). Rheological characteristics and thermal degradation kinetics of beta-carotene in pumpkin puree. *Journal of food engineering*, *76*(4), 538-546.

Ejedegba, B. O., Onyeneke, E. C., & Oviasogie, P. O. (2007). Characteristics of lipase isolated from coconut (*Cocos nucifera linn*) seed under different nutrient treatments. *African Journal of Biotechnology*, *6*(6).

El-Soukkary, F. A. H. (2001). Evaluation of pumpkin seed products for bread fortification. *Plant Foods for Human Nutrition*, *56*(4), 365-384.

Eltaweel, M. A., Rahman, R. N. Z. R. A., Salleh, A. B., & Basri, M. (2005). An organic solvent-stable lipase from *Bacillus sp*. strain 42. *Annals of microbiology*,*55*(3), 187.

Enujiugha, V. N., Thani, F. A., Sanni, T. M., & Abigor, R. D. (2004). Lipase activity in dormant seeds of the African oil bean (*Pentaclethra macrophylla* Benth). *Food chemistry*, *88*(3), 405-410.

E Silva, G. M. D. M., Marques, D. D. A. V., Porto, T. S., Lima Filho, J. L., Teixeira, J. A. C., Pessoa-Júnior, A., & Porto, A. L. F. (2013). Extraction of fibrinolytic proteases from Streptomyces sp. DPUA1576 using PEG-phosphate aqueous two-phase systems.*Fluid Phase Equilibria*, *339*, 52-57.

Ezhilarasi, P. N., Indrani, D., Jena, B. S., & Anandharamakrishnan, C. (2013). Freeze drying technique for microencapsulation of Garcinia fruit extract and its effect on bread quality. *Journal of Food Engineering*, *117*(4), 513-520.

Fan, J., Unoki, H., Kojima, N., Sun, H., Shimoyamada, H., Deng, H., ... & Watanabe, T. (2001). Overexpression of lipoprotein lipase in transgenic rabbits inhibits diet-induced hypercholesterolemia and atherosclerosis.*Journal of Biological Chemistry*, *276*(43), 40071-40079.

Fan, Z., Yue, C., Tang, Y., & Zhang, Y. (2009). Cloning, sequence analysis and expression of bacterial lipase-coding DNA fragments from environment in *Escherichia coli. Molecular biology reports*, *36*(6), 1515-1519.

Ferriol, M., & Picó, B. (2008). Pumpkin and winter squash. In *Vegetables I*(pp. 317-349). Springer New York.

Food and Agriculture Organization (FAO). (2007). FAOSTAT-FAO statistical database. http://faostat.fao.org/site/339/default.aspx. Accessed on 12 January 2016

Fojan, P., Jonson, P. H., Petersen, M. T., & Petersen, S. B. (2000). What distinguishes an esterase from a lipase: a novel structural approach. *Biochimie*, *82*(11), 1033-1041.

Freire, G. D. M., & Castilho, F. L. (2008). Lipases em biocatálise. *Enzimas em biotecnologia: Produção, Aplicação e Mercado. Rio de Janeiro, Interciência*, 369-348.

Freire, M. G., Claudio, A. F. M., Araujo, J. M., Coutinho, J. A., Marrucho, I. M., Lopes, J. N. C., & Rebelo, L. P. N. (2012). Aqueous biphasic systems: a boost brought about by using ionic liquids. *Chemical Society Reviews*,*41*(14), 4966-4995.

Ghaedi, M., Shokrollahi, A., Ahmadi, F., Rajabi, H. R., & Soylak, M. (2008). Cloud point extraction for the determination of copper, nickel and cobalt ions

in environmental samples by flame atomic absorption spectrometry. *Journal of Hazardous Materials*, *150*(3), 533-540.

Ghanem, A., & Ghaly, A. (2004). Immobilization of glucose oxidase in chitosan gel beads. *Journal of applied polymer science*, *91*(2), 861-866.

Gonçalves, E. M., Pinheiro, J., Abreu, M., Brandão, T. R. S., & Silva, C. L. (2007). Modelling the kinetics of peroxidase inactivation, colour and texture changes of pumpkin (*Cucurbita maxima L.)* during blanching. *Journal of Food Engineering*, *81*(4), 693-701.

Gouin, S. (2004). Microencapsulation: industrial appraisal of existing technologies and trends. *Trends in food science & technology*, *15*(7), 330-347.

Guan, G., Sakurai, N., & Kusakabe, K. (2009). Synthesis of biodiesel from sunflower oil at room temperature in the presence of various cosolvents.*Chemical Engineering Journal*, *146*(2), 302-306.

Guillon, F., & Champ, M. (2000). Structural and physical properties of dietary fibres, and consequences of processing on human physiology. *Food Research International*, *33*(3), 233-245.

Gupta, R., Beg, Q., & Lorenz, P. (2002). Bacterial alkaline proteases: molecular approaches and industrial applications. *Applied microbiology and biotechnology*, *59*(1), 15-32.

Gupta, R., Gupta, N., & Rathi, P. (2004). Bacterial lipases: an overview of production, purification and biochemical properties. *Applied microbiology and biotechnology*, *64*(6), 763-781.

Haddar, A., Agrebi, R., Bougatef, A., Hmidet, N., Sellami-Kamoun, A., & Nasri, M. (2009). Two detergent stable alkaline serine-proteases from Bacillus mojavensis A21: Purification, characterization and potential application as a laundry detergent additive. *Bioresource Technology*, *100*(13), 3366-3373.

Hameed, B. H., & El-Khaiary, M. I. (2008). Removal of basic dye from aqueous medium using a novel agricultural waste material: Pumpkin seed hull. *Journal of Hazardous Materials*, *155*(3), 601-609.

Hamilton, M. T., Hamilton, D. G., & Zderic, T. W. (2004). Exercise physiology versus inactivity physiology: an essential concept for understanding lipoprotein lipase regulation. *Exercise and sport sciences reviews*, *32*(4), 161.

Hasan, F., Shah, A. A., & Hameed, A. (2006). Industrial applications of microbial lipases. *Enzyme and Microbial technology*, *39*(2), 235-251.

Hatti-Kaul, R. (2001). Aqueous two-phase systems. *Molecular biotechnology*,*19*(3), 269-277.

Hung, T. C., Giridhar, R., Chiou, S. H., & Wu, W. T. (2003). Binary immobilization of Candida rugosa lipase on chitosan. *Journal of Molecular Catalysis B: Enzymatic*, *26*(1), 69-78.

Ichikawa, T., Yano, Y., Fujita, Y., Kashiwabara, T., & Nagao, K. (2008). The enhancement effect of three sugar alcohols on the fungicidal effect of benzethonium chloride toward Candida albicans. *Journal of dentistry*, *36*(11), 965-968.

Isbilir, S. S., Ozcan, H. M., & Yagar, H. (2008). Some biochemical properties of lipase from Bay Laurel (*Laurus nobilis L.*) seeds. *Journal of the American Oil Chemists' Society*, *85*(3), 227-233.

Iso, M., Chen, B., Eguchi, M., Kudo, T., & Shrestha, S. (2001). Production of biodiesel fuel from triglycerides and alcohol using immobilized lipase.*Journal of Molecular Catalysis B: Enzymatic*, *16*(1), 53-58.

Jirapa, P., Normah, H., Zamaliah, M. M., Asmah, R., & Mohamad, K. (2001). Nutritional quality of germinated cowpea flour (*Vigna unguiculata*) and its application in home prepared powdered weaning foods. *Plant Foods for Human Nutrition*, *56*(3), 203-216.

Jun, H. I., Lee, C. H., Song, G. S., & Kim, Y. S. (2006). Characterization of the pectic polysaccharides from pumpkin peel. *LWT-Food Science and Technology*, *39*(5), 554-561.

Kadkhodaee, R., & Povey, M. J. (2008). Ultrasonic inactivation of Bacillus α-amylase. I. Effect of gas content and emitting face of probe. *Ultrasonics Sonochemistry*, *15*(2), 133-142.

Kandlakunta, B., Rajendran, A., & Thingnganing, L. (2008). Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. *Food Chemistry*, *106*(1), 85-89.

Kaul, A. (2000). The phase diagram. *Aqueous Two-Phase Systems: Methods and Protocols: Methods and Protocols*, 11-21.

Kapranchikov, V. S., Zherebtsov, N. A., & Popova, T. N. (2004). Purification and characterization of lipase from wheat (Triticum aestivum L.) germ.*Applied Biochemistry and Microbiology*, *40*(1), 84-88.

Kawai, K., & Suzuki, T. (2007). Stabilizing effect of four types of disaccharide on the enzymatic activity of freeze-dried lactate dehydrogenase: step by step evaluation from freezing to storage. *Pharmaceutical research*, *24*(10), 1883-1890.

Kim, C. W., & Rha, C. (2000). Phase separation of polyethylene glycol/salt aqueous two-phase systems. *Physics and Chemistry of Liquids*, *38*(2), 181-191.

Klein, B. P., & Kurilich, A. C. (2000). Processing effects on dietary antioxidants from plant foods. *HortScience*, *35*(4), 580-584.

Kronholm, J., Hartonen, K., & Riekkola, M. L. (2007). Analytical extractions with water at elevated temperatures and pressures. *TrAC Trends in Analytical Chemistry*, *26*(5), 396-412.

Lee, Y. K., Chung, W. I., & Ezura, H. (2003). Efficient plant regeneration via organogenesis in winter squash (*Cucurbita maxima Duch.*). *Plant science*,*164*(3), 413-418.

Lee, C. H., Cho, J. K., Lee, S. J., Koh, W., Park, W., & Kim, C. H. (2002). Enhancing β-carotene content in Asian noodles by adding pumpkin powder.*Cereal chemistry*, *79*(4), 593-595.

Leonelli, C., & Mason, T. J. (2010). Microwave and ultrasonic processing: now a realistic option for industry. *Chemical Engineering and Processing: Process Intensification*, *49*(9), 885-900.

Liaquat, M., & Apenten, R. K. (2000). Synthesis of low molecular weight flavor esters using plant seedling lipases in organic media. *Journal of food science*, *65*(2), 295-299.

Lim, T. K. (2012). *Cucurbita moschata*. In *Edible Medicinal And Non-Medicinal Plants* (pp. 266-280). Springer Netherlands.

Loukou, A. L., Gnakri, D., Djè, Y., Kippré, A. V., Malice, M., Baudoin, J. P., & Bi, I. A. (2007). Macronutrient composition of three cucurbit species cultivated for seed consumption in Côte d'Ivoire. *African Journal of Biotechnology*, *6*(5).

Luechau, F., Ling, T. C., & Lyddiatt, A. (2009). Primary capture of high molecular weight nucleic acids using aqueous two-phase systems.*Separation and Purification Technology*, *66*(1), 202-207.

Luque-Garcıa, J. L., & De Castro, M. L. (2003). Ultrasound: a powerful tool for leaching. *TrAC Trends in Analytical Chemistry*, *22*(1), 41-47.

Madhusudhan, M. C., Raghavarao, K. S. M. S., & Nene, S. (2008). Integrated process for extraction and purification of alcohol dehydrogenase from Baker's yeast involving precipitation and aqueous two phase extraction. *Biochemical Engineering Journal*, *38*(3), 414-420.

Malaysian Agricultural Research and Development Institute (MARDI), 2007. Country Report on the State Of Plant Genetic Resources For Food and Agriculture. http://www.fao.org/docrep/013/i1500e/malaysia.pdf. Accessed 15 March 2016

Mason, T. J., & Lorimer, J. P. (2002). Applied sonochemistry. *The uses of power ultrasound in chemistry and processing*, 1-48.

Mayor, L., Cunha, R. L., & Sereno, A. M. (2007). Relation between mechanical properties and structural changes during osmotic dehydration of pumpkin. *Food Research International*, *40*(4), 448-460.

Meng, X. C., Stanton, C., Fitzgerald, G. F., Daly, C., & Ross, R. P. (2008). Anhydrobiotics: The challenges of drying probiotic cultures. *Food Chemistry*,*106*(4), 1406-1416.

Mohamed, M. A., Mohamed, T. M., Mohamed, S. A., & Fahmy, A. S. (2000). Distribution of lipases in the Gramineae. Partial purification and characterization of esterase from Avena fatua. *Bioresource Technology*,*73*(3), 227-234.

Mohammadi, H. S., & Omidinia, E. (2013). Process integration for the recovery and purification of recombinant Pseudomonas fluorescens proline dehydrogenase using aqueous two-phase systems. *Journal of Chromatography B*, *929*, 11-17.

Moing, A. (2000). Sugar alcohols as carbohydrate reserves in some higher plants. *Developments in Crop Science*, *26*, 337-358.

Muralidhar, R. V., Chirumamilla, R. R., Ramachandran, V. N., Marchant, R., & Nigam, P. (2000). Racemic resolution of RS-baclofen using lipase from *Candida cylindracea*. *Mededelingen (Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen)*, *66*(3a), 227-232.

Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2009). *Response surface methodology: process and product optimization using designed experiments* (Vol. 705). John Wiley & Sons.

Nawirska, A., Figiel, A., Kucharska, A. Z., Sokół-Łętowska, A., & Biesiada, A. (2009). Drying kinetics and quality parameters of pumpkin slices dehydrated using different methods. *Journal of Food Engineering*, *94*(1), 14-20.

Negro, C., Tommasi, L., & Miceli, A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technology*,*87*(1), 41-44.

O'donnell, C. P., Tiwari, B. K., Bourke, P., & Cullen, P. J. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in food science & technology*, *21*(7), 358-367.

Ooi, C. W., Tey, B. T., Hii, S. L., Kamal, S. M. M., Lan, J. C. W., Ariff, A., & Ling, T. C. (2009). Purification of lipase derived from *Burkholderia pseudomallei* with alcohol/salt-based aqueous two-phase systems. *Process Biochemistry*, *44*(10), 1083-1087.

Padilha, G. D. S., Santana, J. C. C., Alegre, R. M., & Tambourgi, E. B. (2012). Extraction of lipase from *Burkholderia cepacia* by PEG/Phosphate ATPS and its biochemical characterization. *Brazilian Archives of Biology and Technology*,*55*(1), 7-19.

Palekar, A. A., Vasudevan, P. T., & Yan, S. (2000). Purification of lipase: a review. *Biocatalysis and Biotransformation*, *18*(3), 177-200.

Palomo, J. M., Ortiz, C., Fernández-Lorente, G., Fuentes, M., Guisán, J. M., & Fernández-Lafuente, R. (2005). Lipase–lipase interactions as a new tool to immobilize and modulate the lipase properties. *Enzyme and microbial technology*, *36*(4), 447-454.

Panadero, M., Bocos, C., & Herrera, E. (2006). Relationship between lipoprotein lipase and peroxisome proliferator-activated receptor-α expression in rat liver during development. *Journal of physiology and biochemistry*,*62*(3), 189-198.

Pandey, A., Benjamin, S., Soccol, C. R., Nigam, P., Krieger, N., & Soccol, V. T. (1999). The realm of microbial lipases in biotechnology. *Biotechnology and applied biochemistry*, *29*(2), 119-131.

Paris, H. S. (2015). Germplasm enhancement of *Cucurbita pepo* (pumpkin, squash, gourd: Cucurbitaceae): progress and challenges. *Euphytica*, 1-24.

Passos, H., Trindade, M. P., Vaz, T. S., da Costa, L. P., Freire, M. G., & Coutinho, J. A. (2013). The impact of self-aggregation on the extraction of biomolecules in ionic-liquid-based aqueous two-phase systems. *Separation and Purification Technology*, *108*, 174-180.

Patil, G., & Raghavarao, K. S. M. S. (2007). Aqueous two phase extraction for purification of C-phycocyanin. *Biochemical Engineering Journal*, *34*(2), 156-164.

Pizarro, A. V. L., & Park, E. Y. (2003). Lipase-catalyzed production of biodiesel fuel from vegetable oils contained in waste activated bleaching earth. *Process Biochemistry*, *38*(7), 1077-1082.

Perez, N. E., & Schmalko, M. E. (2009). Convective drying of pumpkin: influence of pretreatment and drying temperature. *Journal of food process engineering*, *32*(1), 88-103.

Polizelli, P. P., Tiera, M. J., & Bonilla-Rodriguez, G. O. (2008). Effect of surfactants and polyethylene glycol on the activity and stability of a lipase from oilseeds of Pachira aquatica. *Journal of the American Oil Chemists' Society*, *85*(8), 749-753.

Pongjanta, J., Naulbunrang, A., Kawngdang, S., Manon, T., & Thepjaikat, T. (2006). Utilization of pumpkin powder in bakery products. *Songklanakarin J. Sci. Technol*, *28*(1), 71-79.

Ponnusamy, S., Zinjarde, S., Bhargava, S., Rajamohanan, P. R., & RaviKumar, A. (2012). Discovering Bisdemethoxycurcumin from Curcuma longa rhizome as a potent small molecule inhibitor of human pancreatic α-amylase, a target for type-2 diabetes. *Food chemistry*, *135*(4), 2638-2642.

Popović, S., Peričin, D., Vaštag, Ž., Popović, L., & Lazić, V. (2011). Evaluation of edible film-forming ability of pumpkin oil cake; effect of pH and temperature. *Food Hydrocolloids*, *25*(3), 470-476.

Porto, T. S., e Silva, G. M., Porto, C. S., Cavalcanti, M. T. H., Neto, B. B., Lima-Filho, J. L., ... & Pessoa, A. (2008). Liquid–liquid extraction of proteases from fermented broth by PEG/citrate aqueous two-phase system. *Chemical Engineering and Processing: Process Intensification*, *47*(4), 716-721.

Prasad, K., & Nath, N. (2002). Comparison of sugarcane juice based beverage optimisation using response surface methodology with Fuzzy method. *Sugar Tech*, *4*(3-4), 109-115.

Preiss-Landl, K., Zimmermann, R., Hämmerle, G., & Zechner, R. (2002). Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. *Current opinion in lipidology*, *13*(5), 471-481.

Raja, S., & Murty, V. R. (2013). Optimization of aqueous two-phase systems for the recovery of soluble proteins from tannery wastewater using response surface methodology. *Journal of Engineering*, *2013*.

Ramakrishnan, A., Pandit, N., Badgujar, M., Bhaskar, C., & Rao, M. (2007). Encapsulation of endoglucanase using a biopolymer Gum Arabic for its controlled release. *Bioresource technology*, *98*(2), 368-372.

Ratanapongleka, K. (2010). Recovery of biological products in aqueous two phase systems. *International Journal of Chemical Engineering and Applications*, *1*(2), 191-198.

Rathi, P., Bradoo, S., Saxena, R. K., & Gupta, R. (2000). A hyper-thermostable, alkaline lipase from Pseudomonas sp. with the property of thermal activation. *Biotechnology Letters*, *22*(6), 495-498.

Rathi, P., Saxena, R. K., & Gupta, R. (2001). A novel alkaline lipase from *Burkholderia cepacia* for detergent formulation. *Process Biochemistry*, *37*(2), 187-192.

Reis, I. A., Santos, S. B., Santos, L. A., Oliveira, N., Freire, M. G., Pereira, J. F., Ventura, S. P. M., Coutinho, J. A. P., Soares, C. M. F. & Lima, Á. S. (2012). Increased significance of food wastes: Selective recovery of added-value compounds. *Food chemistry*, *135*(4), 2453-2461.

Rito-Palomares, M. (2004). Practical application of aqueous two-phase partition to process development for the recovery of biological products. *Journal of Chromatography B*, *807*(1), 3-11.

Rito-Palomares, M., Nunez, L., & Amador, D. (2001). Practical application of aqueous two-phase systems for the development of a prototype process for c-phycocyanin recovery from Spirulina maxima. *Journal of Chemical Technology and Biotechnology*,*76*(12), 1273-1280.

Rosa, P. A. J., Ferreira, I. F., Azevedo, A. M., & Aires-Barros, M. R. (2010). Aqueous two-phase systems: a viable platform in the manufacturing of biopharmaceuticals. *Journal of Chromatography A*, *1217*(16), 2296-2305.

Rowe, E. H., Liedl, F., & Tancibok, K. U. (2001). *U.S. Patent No. 6,284,303*. Washington, DC: U.S. Patent and Trademark Office.

Savchenko, A., Vieille, C., Kang, S., & Zeikus, J. G. (2002). *Pyrococcus furiosus* α-amylase is stabilized by calcium and zinc. *Biochemistry*, *41*(19), 6193-6201.

Sadeghipour, H. R., & Bhatla, S. C. (2003). Light-enhanced oil body mobilization in sunflower seedlings accompanies faster protease action on oleosins. *Plant Physiology and Biochemistry*, *41*(4), 309-316.

Sagiroglu, A., & Arabaci, N. (2005). Sunflower seed lipase: extraction, purification, and characterization. *Preparative Biochemistry and Biotechnology*, *35*(1), 37-51.

Sagoo, G. S., Tatt, I., Salanti, G., Butterworth, A. S., Sarwar, N., van Maarle, M., Jukema, J. W., Wiman, B., Kastelein, J. J. P., Bennet, A. M., de Faire, U., Danesh, J. & Higgins, J. P. T. (2008). Seven lipoprotein lipase gene polymorphisms, lipid fractions, and coronary disease: a HuGE association review and meta-analysis. *American journal of epidemiology*, *168*(11), 1233-1246.

Sammour R. H. A. (2005). Purification and partial characterisation of an acid lipase in germinating lipidbody linseedlings. *Turkish Journal of Botany*,*29*(3), 177-184.

Sana, N. K., Hossin, I., Haque, E. M., & Shaha, R. K. (2004). Identification, purification and characterization of lipase from germinating oil seeds (Brassica napus L.). *Pakistan Journal of Biological Sciences*, *7*(2), 246-252.

Santos, K. C., Cassimiro, D. M., Avelar, M. H., Hirata, D. B., de Castro, H. F., Fernández-Lafuente, R., & Mendes, A. A. (2013). Characterization of the catalytic properties of lipases from plant seeds for the production of

concentrated fatty acids from different vegetable oils. *Industrial Crops and Products*, *49*, 462-470.

Saravanan, S., Rao, J. R., Murugesan, T., Nair, B. U., & Ramasami, T. (2007). Partition of tannery wastewater proteins in aqueous two-phase poly (ethylene glycol)-magnesium sulfate systems: Effects of molecular weights and pH. *Chemical engineering science*, *62*(4), 969-978.

Schindler, J., & Nothwang, H. G. (2006). Aqueous polymer two-phase systems: Effective tools for plasma membrane proteomics. *Proteomics*,*6*(20), 5409-5417.

Seo, J. S., Burri, B. J., Quan, Z., & Neidlinger, T. R. (2005). Extraction and chromatography of carotenoids from pumpkin. *Journal of Chromatography A*,*1073*(1), 371-375.

Senuma, Y., Lowe, C., Zweifel, Y., Hilborn, J. G., & Marison, I. (2000). Alginate hydrogel microspheres and microcapsules prepared by spinning disk atomization. *Biotechnology and bioengineering*, *67*(5), 616-622.

Shah, S., Sharma, S., & Gupta, M. N. (2004). Biodiesel preparation by lipase-catalyzed transesterification of Jatropha oil. *Energy & Fuels*, *18*(1), 154-159.

Shahidan, N., Jaswir, I., Othman, R., Hashim, H., & Zuhanis, Y. (2014). Carotenoid Content in Different Locality of Pumpkin (*Cucurbita moschata*) in Malaysia. *International Journal of Pharmacy and Pharmaceutical Sciences*,*6*(3), 29-32.

Sharma, R., Chisti, Y., & Banerjee, U. C. (2001). Production, purification, characterization, and applications of lipases. *Biotechnology advances*, *19*(8), 627-662.

Show, P. L., Tan, C. P., Anuar, M. S., Ariff, A., Yusof, Y. A., Chen, S. K., & Ling, T. C. (2012). Primary recovery of lipase derived from *Burkholderia cenocepacia* strain ST8 and recycling of phase components in an aqueous two-phase system. *Biochemical Engineering Journal*, *60*, 74-80.

Silva, E. L., dos Santos Roldan, P., & Giné, M. F. (2009). Simultaneous preconcentration of copper, zinc, cadmium, and nickel in water samples by cloud point extraction using 4-(2-pyridylazo)-resorcinol and their determination by inductively coupled plasma optic emission spectrometry.*Journal of Hazardous Materials*, *171*(1), 1133-1138.

Silva, W. O. B., Mitidieri, S., Schrank, A., & Vainstein, M. H. (2005). Production and extraction of an extracellular lipase from the entomopathogenic fungus Metarhizium anisopliae. *Process Biochemistry*,*40*(1), 321-326.

Simpson, R. J. (2010). Stabilization of proteins for storage. *Cold Spring Harbor Protocols*, *2010*(5), pdb-top79.

Souza, R. L., Lima, R. A., Coutinho, J. A., Soares, C. M., & Lima, Á. S. (2015). Novel aqueous two-phase systems based on tetrahydrofuran and potassium phosphate buffer for purification of lipase. *Process Biochemistry*,*50*(9), 1459-1467.

Streitner, N., Voß, C., & Flaschel, E. (2007). Reverse micellar extraction systems for the purification of pharmaceutical grade plasmid DNA. *Journal of biotechnology*, *131*(2), 188-196.

Spelzini, D., Farruggia, B., & Picó, G. (2005). Features of the acid protease partition in aqueous two-phase systems of polyethylene glycol–phosphate: chymosin and pepsin. *Journal of Chromatography B*, *821*(1), 60-66.

Swain, J. F., McCarron, P. B., Hamilton, E. F., Sacks, F. M., & Appel, L. J. (2008). Characteristics of the diet patterns tested in the optimal macronutrient intake trial to prevent heart disease (OmniHeart): options for a heart-healthy diet. *Journal of the American Dietetic Association*, *108*(2), 257-265.

Swarnalatha, V., Esther, R. A., & Dhamodharan, R. (2013). Immobilization of α-amylase on gum acacia stabilized magnetite nanoparticles, an easily recoverable and reusable support. *Journal of Molecular Catalysis B: Enzymatic*, *96*, 6-13.

Tani, T., Uehara, K., Sudo, T., Marukawa, K., Yasuda, Y., & Kimura, Y. (2000). Cilostazol, a selective type III phosphodiesterase inhibitor, decreases triglyceride and increases HDL cholesterol levels by increasing lipoprotein lipase activity in rats. *Atherosclerosis*, *152*(2), 299-305.

Taylor, D.J., Green, N.P.O. and Stout, G. W. (2005). *Biological science.3rd edition* page 8

Teoh, P. L., Mirhosseini, S. H., Mustafa, S., & Manap, M. Y. A. (2011). Tolerance of free and encapsulated probiotics towards heat treatment and high sodium concentration. Journal of Food, Agriculture & Environment, *9*(1), 69-73.

Teotia, M. S., Saxena, A. K., Berry, S. K., & Ahuja, D. K. (2004). Development of Instant Pumpkin Kofta. *Journal of Food Science and Technology*, *41*(6), 703-706.

Thakur, S. (2012). Lipases, its sources, properties and applications: A Review. *International Journal of Scientific & Engineering Research*, *3*(7), 1-29.

Tiwari, G. J., Chiang, M. Y., De Silva, J. R., Song, B. K., Lau, Y. L., & Rahman, S. (2016). Lipase genes expressed in rice bran: LOC_Os11g43510 encodes a novel rice lipase. *Journal of Cereal Science*, *71*, 43-52.

Tobiszewski, M., Mechlińska, A., Zygmunt, B., & Namieśnik, J. (2009). Green analytical chemistry in sample preparation for determination of trace organic pollutants. *TrAC Trends in Analytical Chemistry*, *28*(8), 943-951.

Trabelsi, I., Ayadi, D., Bejar, W., Bejar, S., Chouayekh, H., & Salah, R. B. (2014). Effects of Lactobacillus plantarum immobilization in alginate coated with chitosan and gelatin on antibacterial activity. *International journal of biological macromolecules*, *64*, 84-89.

Tuter, M., Secundo, F., Riva, S., Aksoy, H. A., & Ustun, G. (2003). Partial purification of Nigella sativa L. seed lipase and its application in transesterification reactions. *Journal of the American Oil Chemists' Society*,*80*(1), 43-48.

Villeneuve, P. (2003). Plant lipases and their applications in oils and fats modification. *European Journal of Lipid Science and Technology*, *105*(6), 308-317.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry—A review. *Innovative Food Science & Emerging Technologies*, *9*(2), 161-169.

Walter, H. (Ed.). (2012). *Partitioning in aqueous two–phase system: theory, methods, uses, and applications to biotechnology*. Elsevier.

Wanasundara, P. K. J. P. D., Wanasundara, U. N., & Shahidi, F. (2001). Lipolytic activity of enzymes from germinating seeds of sesame (Sesamum indicum L.). *Journal of Food Lipids*, *8*(2), 75-84.

Wang, D. Q., Hey, J. M., & Nail, S. L. (2004). Effect of collapse on the stability of freeze-dried recombinant factor VIII and α-amylase. *Journal of pharmaceutical sciences*, *93*(5), 1253-1263.

Weng, W. L., Liu, Y. C., & Lin, C. W. (2001). Studies on the optimum models of the dairy product Kou Woan Lao using response surface methodology.*Asian Australasian Journal of Animal Sciences*, *14*(10), 1470-1476.

Wenli, Y., Yaping, Z., Jingjing, C., & Bo, S. (2004). Comparison of two kinds of pumpkin seed oils obtained by supercritical CO_2 extraction. *European journal of lipid science and technology*, *106*(6), 355-358.

Whelehan, M., von Stockar, U., & Marison, I. W. (2010). Removal of pharmaceuticals from water: Using liquid-core microcapsules as a novel approach. *water research*, *44*(7), 2314-2324.

Xia, X., Wang, Y. H., Yang, B., & Wang, X. (2009). Wheat germ lipase catalyzed kinetic resolution of secondary alcohols in non-aqueous media.*Biotechnology letters*, *31*(1), 83-87.

Yan, P. T., Tan, C. L., Yusoff, K., Wen, S. T., & Beng, T. T. (2005). Comparative evaluation of three purification methods for the nucleocapsid protein of Newcastle disease virus from Escherichia coli homogenates. *The Journal of Microbiology*, *43*(3), 295-300.

Yeşiloğlu, Y., & Başkurt, L. (2008). Partial purification and characterization of almond seed lipase. *Preparative biochemistry & biotechnology*, *38*(4), 397-410.

Yilmaz, E., Sezgin, M., & Yilmaz, M. (2010). Enantioselective hydrolysis of rasemic naproxen methyl ester with sol–gel encapsulated lipase in the presence of sporopollenin. *Journal of Molecular Catalysis B: Enzymatic*,*62*(2), 162-168.

Younis, Y. M. H., Ghirmay, S., & Al-Shihry, S. S. (2000). African *Cucurbita pepo L.:* properties of seed and variability in fatty acid composition of seed oil. *Phytochemistry*, *54*(1), 71-75.

Zhang, A., Gao, R., Diao, N., Xie, G., Gao, G., & Cao, S. (2009). Cloning, expression and characterization of an organic solvent tolerant lipase from *Pseudomonas fluorescens* JCM5963. *Journal of Molecular Catalysis B: Enzymatic*, *56*(2), 78-84.

Zhang, H. Y., Wang, X., Ching, C. B., & Wu, J. C. (2005). Experimental optimization of enzymic kinetic resolution of racemic flurbiprofen.*Biotechnology and applied biochemistry*, *42*(1), 67-71.

Zhang, M., Hu, P., Liang, Q., Yang, H., Liu, Q., Wang, Y., & Luo, G. (2007). Direct process integration of extraction and expanded bed adsorption in the recovery of crocetin derivatives from Fructus gardenia. *Journal of Chromatography B*, *858*(1), 220-226.

Zhou, Q. Z., & Chen, X. D. (2001). Effects of temperature and pH on the catalytic activity of the immobilized β-galactosidase from Kluyveromyces lactis. *Biochemical Engineering Journal*, *9*(1), 33-40.

Zhao, X., Xie, X., & Yan, Y. (2011). Liquid–liquid equilibrium of aqueous two-phase systems containing poly (propylene glycol) and salt ((NH 4) 2 SO 4, MgSO 4, KCl, and KAc): experiment and correlation. *Thermochimica Acta*,*516*(1), 46-51.

Zhong, H., ming Zeng, G., Yuan, X. Z., yan Fu, H., Huang, G. H., & Ren, F. Y. (2007). Adsorption of dirhamnolipid on four microorganisms and the effect on cell surface hydrophobicity.*Applied microbiology and biotechnology*, *77*(2), 447-455.

Zohdi, N. K., & Amid, M. (2013). Optimization of extraction of novel pectinase enzyme discovered in Red Pitaya (*Hylocereus polyrhizus*) peel. *Molecules*,*18*(11), 14366-14380.

PUBLICATION

Mehrnoush Amid, Mohd Yazid Manap, **Muhaini Hussin**, and Shuhaimi Mustafa. "A Novel Aqueous Two Phase System Composed of Surfactant and Xylitol for the Purification of Lipase from Pumpkin (*Cucurbita moschata*) Seeds and Recycling of Phase Components. " Molecules , 20 (6), (2015): 11184-11201

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : _____

TITLE OF THESIS / PROJECT REPORT :

EXTRACTION, PURIFICATION, MICROENCAPSULATION AND CHARACTERIZATION OF LIPASE FROM PUMPKIN (*Cucurbita moschata* DUCHESNE EX POIR.) SEED

NAME OF STUDENT : MUHAINI BINTI MOHD HUSSIN

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from_____ until _____ (date) (date)

Approved by:

_____	_____
(Signature of Student)	(Signature of Chairman of Supervisory Committee)
New IC No/ Passport No.:	Name:
Date :	Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]