EXTRACTION, PURIFICATION AND CHARACTERIZATION OF PECTINASE FROM GUAVA (*Psidium guajava L.*) PEEL

By

FARA SYAZANA BINTI AHMAD MURSHID

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

March 2017

FSTM 2017 3

COPYRIGHT

DEDICATION

I dedicate this thesis to the love of my life, especially my dear father, Ahmad Murshid Abu and mother, Aini Md Jadi. To my dear brothers and sister; along, angah and adik, thank you for the endless love, support and inspiration through all these years. To my dear Keisha Adam and Iman, the source of my happiness.

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 AND CHARACTERIZATION OF PECTINASE FROM GUAVA (*Psidium guajava* L.) PEEL

By

FARA SYAZANA BINTI AHMAD MURSHID

March 2017

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

Pectinase brakes down pectin which is a polysaccharide that commonly found in plant cell wall. Mainly, enzymes are easily degraded with inappropriate method of extraction. Hence, it is important to employ an inexpensive, simple and efficient method of extraction. In this study, pectinase was extracted from guava *(Psidium guajava)* peel with ultrasound assisted extraction. The main effects namely sonication time, ultrasound temperature, pH of buffer and buffer to sample (B/S) ratio for optimization of the extraction were investigated. The optimum extraction condition was achieved at 20 min sonication time, 40 °C ultrasound temperature, at pH 5.0, using a 4:1 mL/g buffer to sample ratio. Conventional methods of purification are multistep, tedious and expensive. Therefore, the development of cost-effective, highly efficient and environmental friendly procedure for the purification of pectinase with desirable properties is considered essential. Subsequently, the potential application of aqueous two-phase system (ATPS) with Triton X-100 and sorbitol in the purification of pectinase from guava peel was demonstrated at laboratory scale. In this study, the effect of the main important parameters such as Tie Line Length (TLL), crude loads and pH on purification of the enzyme were investigated. The experimental results indicated that the pectinase was partitioned into surfactant-rich top phase, and the impurities were partitioned into the sorbitol-rich bottom phase with the novel method involving an ATPS composed of 25.0% (w/w) Triton X-100 and 26.0% (w/w) sorbitol at 50.2% of the TLL, 20% (w/w) of crude load and at pH 6.0. Based on the results, the calculated purification factor for the pectinase was 15.2 and the yield obtained was 98.3% for purified pectinase from guava peel. It was demonstrated that the phase components, Tie Line Length (TLL), crude loads and pH influenced the pectinase partitioning. This study proved that ATPS can be exploited as a successful, inexpensive and effective method for purification and recovery of the enzyme from a low-cost source with potential industrial application and alternative to the traditional ATPS. Characterization of the purified enzyme was performed to evaluate the stability of pectinase in different conditions. Characterization of the purified enzyme showed that pectinase extracted

from guava peel was stable with the presence of some metal ions, inhibitors, surfactants and oxidizing agents. Metals ion such as K^{+}, Ba^{+}, Mg^{2+}, Na^{+} and Mn^{2+} enhanced pectinase activity. Meanwhile, pectinase showed extreme stability with regards to surfactant and inhibitor including Triton X-100, Tween-80 and EDTA. The molecular weight of the pectinase was estimated to be 24.4 kDa based on SDS-PAGE. Therefore, it can be concluded that the enzyme with unique characteristics could be obtained from natural and cost-effective source and potentially contributed in the industrial applications including food and beverages, textile, paper, waste water treatment and other biotechnological applications.

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

PENGEKSTRAKAN, PENULENAN DAN PENCIRIAN PEKTINASE DARI KULIT JAMBU BATU (*Psidium guajava* L.)

Oleh

FARA SYAZANA BINTI AHMAD MURSHID

Mac 2017

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

Pektinase menguraikan pektin yang merupakan polisakarida yang biasanya terdapat di dalam sel dinding tumbuhan. Proses pengekstrakan yang tidak sesuai boleh menyebabkan perencatan enzim. Oleh itu, adalah penting untuk mengambil kira kaedah pengekstrakan yang murah, mudah dan berkesan. Dalam kajian ini, pektinase telah diekstrak daripada kulit jambu batu (*Psidium guajava*) dengan menggunakan teknik bantuan Ultrasonik. Antara faktor-faktor utama dalam mengoptimumkan pengekstrakan telah dikaji antaranya adalah suhu, masa pengekstrakan, pH larutan dapar dan nisbah larutan dapar kepada sampel (B/S). Pengekstrakan optimum telah dicapai pada 20 minit masa sonikasi, suhu 40 °C, pada pH 5.0, dan menggunakan nisbah 4:1 mL/g larutan buffer kepada sampel. Penulenan enzim secara konvensional merupakan proses yang langkah-berganda, rumit dan memakan kos. Oleh itu, pengunaan prosedur penulenan yang kos-efektif, efisien, dan mesra alam untuk ciri-ciri pektinase yang wajar adalah penting. Selepas itu, aplikasi yang berpotensi untuk penulenan pektinase daripada kulit jambu batu iaitu Sistem Dua Fasa Berakua (SDFB) telah digunakan bersama surfaktan (Triton X-100) dan sorbitol dalam skala makmal. Dalam kajian ini, kesan parameter utama dalam penulenan enzim seperti Panjang Garis Ikatan (PGI), muatan mentah dan pH telah disiasat. Dapatan eksperimen menunjukkan bahawa pektinase telah terbahagi kepada fasa kaya-surfaktan dan segala bendasing telah terbahagi ke fasa dibawah iaitu fasa kaya-sorbitol dengan kaedah novel melibatkan DFB terdiri daripada 25.0% (w/w) Triton X-100 dan 26.0% (w/w) sorbitol di 50.2% PGI, muatan mentah sebanyak 20% (w/w) dan pada pH 6.0. Berdasarkan keputusan yang didapati, faktor penulenan yang diperoleh bagi pektinase tulen dari jambu batu adalah 15.2 dan kadar hasil adalah 98.3%. Ia telah menunjukkan bahawa Panjang Garis Ikatan (PGI), muatan mentah dan pH mempengaruhi pembahagian pektinase. Kajian ini membuktikan bahawa DFB boleh dieksploitasi sebagai kaedah yang berjaya, jimat, dan berkesan untuk penulenan dan pemulihan enzim yang berharga dari sumber yang ber-kos rendah yang mempunyai potensi untuk aplikasi industri dan juga alternatif kepada tradisional DFB. Pencirian enzim tulen telah dilakukan untuk menilai kestabilan

pektinase dalam keadaan yang berbeza. Melalui pencirian enzim tulen ini, didapati pektinase dari kulit jambu batu adalah stabil dengan kehadiran beberapa ion logam, perencat, surfaktan dan agen pengoksidaan. Ion logam seperti K^{+}, Ba^{+}, Mg^{2+}, Na^{+} dan Mn^{2+} meningkatkan lagi aktiviti enzim. Manakala, pektinase menunjukan kestabilan yang tinggi dengan kehadiran surfaktan dan perencat termasuk Triton X-100, Tween-80 dan EDTA. Berat molekul pectinase dari jambu batu ini dianggarkan 24.4 kDa melalui SDS-PAGE. Dapat disimpulkan bahawa enzim dengan ciri-ciri unik boleh diperolehi dari sumber semula jadi dan kos-efektif dan berpotensi menyumbang dalam aplikasi industri seperti industri makanan dan minuman, tekstil, kertas, rawatan sisa air dan juga aplikasi bioteknologi yang lain.

ACKNOWLEDGEMENTS

First and foremost, with the highest gratitude beyond everything, I presumed my thanks to Allah SWT, the Almighty that with His will, blessings that He bestowed upon me that I am able to finish my master thesis, Alhamdulillah.

I would like to give my profoundest gratitude to my supervisor, Associate Professor Dr. Mehrnoush Amid, with her unreserved help and guidance throughout my master journey. I would also like to thank other member of the committee, especially Professor Dato' Dr. Mohd Yazid Abdul Manap with his continuous support, patience and inspiring words in my hardest time and Professor Nazamid Saari for his advices, support and guidance. Not to forget to all the staff involved directly or indirectly. The time, knowledge and guidance are highly appreciated and to them all, I owe a debt of gratitude.

I would also like to extend my boundless love and appreciation to my parents; Ahmad Murshid Abu and Aini Md Jadi, my siblings and families, that with their love, support and encouragement and not to mention emotional and financial support that I could never pay, infinite thank you to all of you. Love you all to the moon and back.

Not to forget, my warmest gratitude to all my friends that always been through thick and thin. I am glad to have you all here and you guys know who you are especially Ima, Aisyah, Husna, Muhaini. Farhana and Dila. Lastly, to everyone that involved directly or indirectly in this journey, He must have sent you all for a reason. Alhamdulillah, and a very big thank you for every single thing.

I certify that a Thesis Examination Committee has met on……. to conduct the final examination of Fara Syazana Binti Ahmad Murshid on her thesis entitled "EXTRACTION, PURIFICATION AND CHARACTERIZATION OF PECTINASE FROM GUAVA (*Psidium guajava L.*) PEEL" 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:

Anis Sobirin Binti Meor Hussin, PhD
Professor Madya
Jabatan Teknologi Makanan
Fakulti Sains dan Teknologi Makanan
Universiti Putra Malaysia
(Pengerusi)

Chong Gun Hean, PhD
Professor Madya
Jabatan Teknologi Makanan
Fakulti Sains dan Teknologi Makanan
Universiti Putra Malaysia
(Pemeriksa Dalam)

Alfi Khatib, PhD
Professor Madya
Jabatan Farmaseutikal Kimia
Fakulti Farmasi
Universiti Islam Antarabangsa Malaysia
(Pemeriksa Luar)

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

Date:

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of 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 Abdul Manap, PhD
Professor
Faculty of Food Science and Technology/ Halal Products Research Institute
Universiti Putra Malaysia
(Member)

Nazamid Saari, PhD
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 Dean

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 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: Fara Syazana Binti Ahmad Murshid , _____

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) were 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 Abdul Manap

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Nazamid Saari

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ATPS	Aqueous Two-Phase System
BSA	Bovine Serum Albumin
DNS	Dinitrosalicyclic acid
DTAB	Dodecyl Trimethyl Ammonium Bromide
DTNB	5,5'-Dithiobis(2-nitrobenzoic acid)
EC	Enzyme Commission
EDTA	Ethylenediaminetetraacitic Acid
kDa	Kilodaltons
LSD	Least Significant Difference
Mt	Metric ton
MW	Molecular weight
PEG	Polyethylene Glycol
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
kcal	kilocalories
inch	inches
m	meter
M	molarity

minutes	min
mg	milligram
ml	milliliter
mm	millimeter
mM	millimole
µmol	micromol
µml	micromilliliter
nm	nanometer
kV	kilovolt
U	Unit
v	volume
w	weight
I.U.	International Units
kHz	kiloHertz
h	hour

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Guava is well-known as one of the important tropical fruits in many countries worldwide. Belongs to *Myrtaceae* family, guava is native in tropical America, currently distributed in tropical and subtropical continents including South-east Asia (El-Ahmady et al., 2013; Martin, 1984). In Malaysia, guava has been cultivated commercially in Perak, Johor, Melaka, Selangor, Negeri Sembilan, Pahang, Perlis and Penang (Kwee & Chong, 1990). Noteworthy, the total crop acreage in 2006 is 1992 hectares, with an average yield of 16.8 tons/hectare and the production is estimated to be RM33.6 million (Ariff & Lin, 2008).

Pectinase are heterogeneous enzymes that catalyze pectin, the polysaccharides in plants into simpler molecules like polygalacturonic acid (Kumar et al., 2012). Pectinase has been one of the most forthcoming enzymes in the commercial sectors, biotechnologies, and industries (Kashyap et al., 2001). Previous report stated that the global food enzymes sales accounted to 25% for pectinase (Jayani et al., 2005). Pectinase has diverse applications including food and beverage industries (Sandri et al., 2011; Nur' Alia et al., 2010; Abdullah et al., 2007), oil extraction and recovery (Demir, et al., 2001), coffee, tea and wine productions (Ibrahim et al., 2013), textile industries (Rajendran et al., 2011), paper and pulp industries (Reid & Richard, 2000), production of animal feed and also being used in waste water treatment (Hoondal et al, 2000).

Ultrasonic assisted extraction (UAE) has drawn increasing attention in numerous studies in the recent years as the methods for extraction of bioactive components in plant tissues (Rouhani et al., 2009; Djilani et al., 2006) and nowadays, ultrasound is widely acknowledged to stimulate various processes of extraction (Cares et al., 2010). The recognition of UAE in the application of various industrial processes compared to other conventional methods of extraction such as maceration, reflux, decoction, infusion and others are due to its advantages in limiting the cost and consumption of time, chemicals, energy, reduces effluent and also minimizing the degradation (Bagherian et al., 2011; Rostagno et al., 2003).

Aqueous two-phase system (ATPS) has been broadly used for extraction of protein, separation and purification of macromolecules, cells, and organelles. Aqueous two-phase systems mainly are mixture of two immiscible solutions typically polymer and salt such as polyethyleneglycol and sulphate, phosphate or citrate salt (da Silva et al., 2015; Goja et al., 2013), mixture of two different polymers with common polymers are polyethylene glycol with different molecular weight (Kulaguin-Chicaroux & Zeiner, 2014), salt and alcohol (Ooi et al., 2009), mixture of two electrolytes (Zafarani-Moattar & Hamzehzadeh, 2007) or polymer and electrolyte (de Lemos et

al., 2011) in water above critical concentration. ATPS can be considered as a potential method to overcome the drawback in chromatography with numerous advantages including low of cost, toxicity and low interfacial tension, high water content, provide friendly environment for separation and purification, easily recycled and scaled up with good operational characteristics (da Silva et al., 2015; Rodrigues et al., 2013; Johansson et al., 2011; Asenjo & Andrews, 2011).

1.2 Problem Statements

Malaysia is still facing the major obstacle to produce and commercialize the enzymes for industrial applications due to the high production cost especially high capital investment (Ibrahim, 2013). However, the demand of the enzymes is subjected to increase annually (Li et al., 2012). Besides that, the industry now demands fast, economic upstream and downstream processes to extract and purify biomolecules with higher yield and purity (Dutra-Molino et al., 2014; Gupta et al., 2002).

Enzymes are subtle to changes in pH, ionic strength, temperature, substrate concentration, and nature of salts presents to the point that it can lead changes in their morphology and also denaturation (Scopes, 2002). Hence, it is important to screen through appropriate method of extraction that able to extract the targeted compounds, increase the selectivity of the analytical methods, increase sensitivity of the bioassay and the concentration of the targeted compounds in an environmental friendly manner, reduced time, labor and cost as well as better yield and quality.

Expensive, time-consuming, difficult to scale up and higher cost with lower yield are the main problems associated with the conventional method for the recovery of the enzyme from fruits (Ooi et al. 2009). The steps include clarification and concentration, primary contaminant removal, intermediate purification, and finally polishing (Goja et al., 2013; Prince & Smith, 2004). In industrial processes, about 70% of the final costs were spent in enzyme purification regardless that high purity of enzyme is not essential in industrial processes, contributed by the chromatographic separation techniques (Jaramillo et al., 2013). Although several chromatographic steps are typically used as purification schemes for protein, each step increases operational cost, processing time and also product losses. Therefore, the development of new separation technology that decrease enzyme purification costs and improve yield is an indispensable prerequisite to expand the market for industrial enzymes and proteins.

1.3 Significance of Study

Studies reported that guava peel has never been used commercially despite the presence of useful bioactive components and; it is still ended up as waste product (Marina & Noriham, 2014). According to Lee et al. (2010), currently, only the edible part of the guava is processed into many products while its peel, is not utilized commercially and discarded as waste material (Lee et al., 2010). Recent

investigation stated that guava peel can be an effective source of antioxidants (Contreras-Calderon et al., 2011; Jiménez-Escrig, 2001; Zulkifli, 2010). Besides that, there is also study indicated the presence of protease in guava peel (Chitturi et al., 2013) and according to Singh et al. (2014), guava peel contains significant amount of pectin. Until now, research on guava peel as a natural source of pectinase is still scarce.

The aim of this study is to optimize the operational condition for extraction of pectinase from guava peel by optimizing the extraction conditions that prevent denaturation of the desired enzyme. Furthermore, the increase in demand for a quick and easy recovery method with improved yield and purity for purification of pectinase especially in industries as well as maintaining the pectinase activity and stability throughout the process are the fundamentals of this research. Enzymes are definitely unique based on their source, therefore identifying and characterizing the extracted pectinase properties will help in understanding its function and best condition for its future usage and novelty.

1.4 Objectives

The general objective of this study is to investigate the effect of extraction and purification of pectinase from guava *(Psidium guajava*) peel as well as to characterize the purified pectinase properties. The specific objectives of this study are:

1. To optimize the ultrasound assisted extraction condition of pectinase from guava *(Psidium guajava)* peel;
2. To develop and optimize the purification procedure of pectinase enzyme from guava *(Psidium guajava)* peel with Aqueous Two-Phase System (ATPS);
3. To characterize the pectinase properties from the guava *(Psidium guajava)* peel.

REFERENCES

Abbott, N. L., Blankschtein, D., Hatton, T. A., (1993). Protein partitioning in two-phase aqueous polymer systems. 5. Decoupling of the effects of protein concentration, salt type, and polymer molecular weight. *Macromolecules,* 26: 825–828.

Abdullah, A. L., Sulaiman, N. M., Aroua, M. K., & Noor, M. M. M. (2007). Response surface optimization of conditions for clarification of carambola fruit juice using a commercial enzyme. *Journal of Food Engineering*, 81(1): 65-71.

Agasøster, T. (1998). Aqueous two-phase partitioning sample preparation prior to liquid chromatography of hydrophilic drugs in blood. *Journal of Chromatography B: Biomedical Sciences and Applications*, 716(1): 293-298.

Ahmed, I., Zia, M. A., Hussain, M. A., Akram, Z., Naveed, M. T., & Nowrouzi, A. (2016). Bioprocessing of citrus waste peel for induced pectinase production by *Aspergillus niger;* its purification and characterization. *Journal of Radiation Research and Applied Sciences*, 9(2): 148-154.

Akhter, N., Morshed, M. A., Uddin, A., Begum, F., Sultan, T., & Azad, A. K. (2011). Production of pectinase by *Aspergillus niger* cultured in solid state media. *International Journal of Biosciences*, 1(1): 33-42.

Akintobi, A. O., Oluitiola, P. O., Olawale, A. K., Odu, N. N., & Okonko, I. O. (2012). Production of pectinase enzymes system in culture filtrates of *Penicillium variabile Sopp*. *Natural Sciences*, 10: 99-109.

Alaña, A., Lama, M. J., & Serra, J. L. (1991). Purification and some properties of the pectin lyase from *Penicillium italicum*. *FEBS Letters*, 280(2): 335.

Albertsson PA. (1956). Chromatography and Partition of Cells and Cell Fragments. *Nature*, 177(4513): 771-774.

Albertsson, P. A. (1986). *Partition of cell particles and macromolecules.* In: Separation and purification of biomolecules, cell organelles, membranes, and cells in aqueous polymer two-phase systems and their use in biochemical analysis and biotechnology. New York: Wiley.

Ali, S. M., Ling, T. C., Muniandy, S., Tan, Y. S., Raman, J., & Sabaratnam, V. (2014). Recovery and partial purification of fibrinolytic enzymes of *Auricularia polytricha (Mont.) Sacc* by an aqueous two-phase system. *Separation and Purification Technology*, 122: 359-366.

Amid, M., Tan, C. P., Mirhosseini, H., Aziz, N. A., & Ling, T. C. (2011). Optimisation of serine protease extraction from mango peel *(Mangifera Indica Cv. Chokanan). Food chemistry*, 124(2): 666-671.

Andrade, M. V. V. D., Delatorre, A. B., Ladeira, S. A., & Martins, M. L. L. (2011). Production and partial characterization of alkaline polygalacturonase secreted by thermophilic *Bacillus sp*. SMIA-2 under submerged culture using pectin and corn steep liquor. *Food Science and Technology (Campinas)*, 31(1): 204-208.

Anem, M. (October, 2012). Guava varieties in Malaysia [Senior Agronomist, Horticulture Division]. Retrived from http://animagro.blogspot. my/2012/12/ guava-varieties-in-malaysia.html.

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.

Antov, M. G., & Peričin, D. M. (2001a). Production of pectinases by Polyporus quamosus in aqueous two-phase system. *Enzyme and Microbial Technology*, 28(4): 467-472.

Antov, M. G., Peričin, D. M., & Dimić, G. R. (2001). Cultivation of Polyporus squamosus for pectinase production in aqueous two-phase system containing sugar beet extraction waste. *Journal of Biotechnology*, 91(1): 83-87.

Antov, M., & Omorjan, R. (2009). Pectinase partitioning in polyethylene glycol 1000/Na_2SO_4 aqueous two-phase system: statistical modeling of the experimental results. *Bioprocess and Biosystems Engineering*, 32(2): 235-240.

Anvari, M. (2015). Extraction of lipase from *Rhizopus microsporus* fermentation culture by aqueous two-phase partitioning. *Biotechnology & Biotechnological Equipment*, 29(4): 723-731.

Arias, C. R., & Burns, J. K. (2002). A pectinmethylesterase gene associated with a heat-stable extract from citrus. *Journal of Agricultural andFood Chemistry*, 50(12): 3465-3472.

Ariff, E. E. E., & Lin, R. M. (2008). Ekonomi pengeluaran jambu batu (Economic production of guava). *Economic Technology and Management Review*. 3: 1-11.

Arijit, D., Sourav, B., Naimisha, R., & Rajan, S. S. (2013). Improved production and purification of pectinase from Streptomyces sp. GHBA10 isolated from Valapattanam mangrove habitat, Kerala, India. *International Research Journal of Biological Sciences*, 2(3): 16-22.

Arijit, D., Sourav, B., Naimisha, R., & Rajan, S. S. (2013). Improved production and purification of pectinase from *Streptomyces sp.* GHBA10 isolated from Valapattanam mangrove habitat, Kerala, India. *International Research Journal of Biological Sciences*, 2(3): 16-22.

Arya, V., Thakur, N., & Kashyap, C. P. (2012). Preliminary phytochemical analysis of the extracts of psidium leaves. *Journal of Pharmacognosy and Phytochemistry*, 1(1): 1-5.

Asad, S., Torabi, S. F., Fathi-Roudsari, M., Ghaemi, N., & Khajeh, K. (2011). Phosphate buffer effects on thermal stability and H_2O_2-resistance of horseradish peroxidase. *International Journal of Biological Macromolecules*, 48(4): 566-570.

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.

Ashokkumar, M., Lee, J., Kentish, S., & Grieser, F. (2007). Bubbles in an acoustic field: an overview. *Ultrasonics Sonochemistry*, 14(4): 470-475.

Azevedo, A. M., Gomes, A. G., Rosa, P. A., Ferreira, I. F., Pisco, A. M., & Aires-Barros, M. R. (2009). Partitioning of human antibodies in polyethylene glycol–sodium citrate aqueous two-phase systems. *Separation and Purification Technology*, 65(1): 14-21.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: a review. *Journal of Food Engineering*, 117(4): 426-436.

Bach, E., Sant'Anna, V., Daroit, D. J., Corrêa, A. P. F., Segalin, J., & Brandelli, A. (2012). Production, one-step purification, and characterization of a keratinolytic protease from *Serratia marcescens* P3. *Process Biochemistry*, 47(12): 2455-2462.

Badwaik, L. S., Borah, P. K., & Deka, S. C. (2015). Optimization of microwave assisted extraction of antioxidant extract from *Garcinia pedunculata Robx*. *Separation Science and Technology*, 50(12): 1814-1822.

Bagherian, H., Ashtiani, F. Z., Fouladitajar, A., & Mohtashamy, M. (2011). Comparisons between conventional, microwave-and ultrasound-assisted methods for extraction of pectin from grapefruit. *Chemical Engineering and Processing: Process Intensification*, 50(11): 1237-1243.

Baladhandayutham, S. & Thangavelu, V. (2010) Optimization and kinectics of pectinase enzyme using *Aspergillus niger* by solid-state fermentation. *Indian Journal of Science and Technology*, 3: 867-870.

Balestri, E., & Cinelli, F. (2001). Isolation and cell wall regeneration of protoplasts from *Posidoniaoceanica* and *Cymodoceanodosa*. *Aquatic Botany*, 70(3): 237-242.

Banu, A. R., Devi, M. K., Gnanaprabhal, G. R., Pradeep, B. V., & Palaniswamy, M. (2010). Production and characterization of pectinase enzyme from

Penicillium chrysogenum. *Indian Journal of Science and Technology*, 3(4): 377-381.

Barbin, D. F., Natsch, A., & Müller, K. (2011). Improvement of functional properties of rapeseed protein concentrates produced via alcoholic processes by thermal and mechanical treatments. *Journal of Food Processing and Preservation*, 35(3): 369-375.

Barrett, D. M., Somogyi, L., & Ramaswamy, H. S. (Eds.). (2004). *Processing Fruits: Science and Technology*. US: CRC Press.

Barron, J. J., Ashton, C., & Geary, L. (2006). The effects of temperature on pH measurement. In: *57th Annual Meeting of the International Society of Electrochemistry, Edinburgh, Technical Services Department, Reagecon Diagnostics Ltd, Ireland.*

Bartley, I. M. (1978). Exo-polygalacturonase of apple. *Phytochemistry*, 17(2): 213-216.

Basu, S., Ghosh, A., Bera, A., Saha, M. N., Chattopadhyay, D., & Chakrabarti, K. (2008). Thermodynamic characterization of a highly thermoactive extracellular pectate lyase from a new isolate *Bacillus pumilus* DKS1. *Bioresource Technology*, 99(17): 8088-8094.

Beijerinck, M. W. (1896). Über eine Eigentümlichkeit der löslichen Stärke.*Centr-Bl. f. Bakter. u. Parasitenk*, *2*: 698-699.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Biochemistr*y. New York: W. H. Freeman and Company, 79-91.

Berggren, K., Johansson, H. O., & Yjerneld, F. (1995). Effects of salts and the surface hydrophobicity of proteins on partitioning in aqueous two-phase systems containing thermoseparating ethylene oxide-propylene oxide copolymers. *Journal of Chromatography A*, 718(1): 67-79.

Bertagnolli, S. M. M., Silveira, M. L. R., Fogaça, A. D. O., Umann, L., & Penna, N. G. (2014). Bioactive compounds and acceptance of cookies made with guava peel flour. *Food Science and Technology (Campinas)*, 34(2): 303-308.

Beşel, E. (2003). *Use of Triton X-114 aqueous two phase system for recovery of mushroom (Agaricus bisporus) polyphenoloxidase*. PhD Thesis, Middle East Technical University.

Bhat, S. A., & Singh, E. R. (2014). Extraction and characterization of pectin from guava fruit peel. *International Journal of Research in Engineering & Advanced Technology*, 2(3): 1-7.

Bhavsar, K., Kumar, V. R., & Khire, J. M. (2012). Downstream processing of extracellular phytase from *Aspergillus niger:* Chromatography process vs.

aqueous two phase extraction for its simultaneous partitioning and purification. *Process Biochemistry*, 47(7): 1066-1072.

Bibi, N., Ali, S., & Tabassum, R. (2016). Statistical optimization of pectinase biosynthesis from orange peel by *Bacillus licheniformis* using submerged fermentation. *Waste and Biomass Valorization*, 7(3): 467-481.

Birch, G. G., Blakebrough, N., & Parker, K. J. (2012). *Enzymes and food processing*. New York: Springer Science & Business Media.

Biz, A., Farias, F. C., Motter, F. A., de Paula, D. H., Richard, P., Krieger, N., & Mitchell, D. A. (2014). Pectinase activity determination: an early deceleration in the release of reducing sugars throws a spanner in the works!. *PloS one*, 9(10): e109529.

Blom, H. (2012). *Purification process for complex biomacromolecules.* PhD Thesis, Upsala University.

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.

Bourton, E. C. (2008). *Countercurrent chromatography of proteins using aqueous two-phase systems*, PhD Thesis, Brunel University School of Engineering and Design.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2): 248-254.

Brady, C. J., Meldrum, S. K., McGlasson, W. B., & Ali, Z. M. (1983). Differential accumulation of the molecular forms of polygalacturonase in tomato mutants. *Journal of Food Biochemistry*, 7(1): 7-14.

Bucar, F., Wube, A., & Schmid, M. (2013). Natural product isolation–how to get from biological material to pure compounds. *Natural Product Reports*, 30(4): 525-545.

Bulduk, I., Gezer, B., & Cengiz, M. (2015). Optimization of Ultrasound-Assisted Extraction of Morphine from Capsules of Papaver somniferum by Response Surface Methodology. *International Journal of Analytical Chemistry*, 2015: 1-8.

Burden, D. W. (2012). Guide to the disruption of biological samples. *Random Primers*, 12: 1-25.

Çam, M., & İçyer, N. C. (2015). Phenolics of pomegranate peels: extraction optimization by central composite design and alpha glucosidase inhibition potentials. *Journal of Food Science and Technology*, 52(3): 1489-1497.

Cameron, R. G., Savary, B. J., Hotchkiss, A. T., & Fishman, M. L. (2005). Isolation, characterization, and pectin-modifying properties of a thermally tolerant pectin methylesterase from *Citrus sinensis var*. Valencia. *Journal of Agricultural and Food Chemistry*, 53(6): 2255-2260.

Cameron, R. G., Savary, B. J., Hotchkiss, A. T., Fishman, M. L., Chau, H. K., Baker, R. A., & Grohmann, K. (2003). Separation and characterization of a salt-dependent pectin methylesterase from *Citrus sinensis var*. Valencia fruit tissue. *Journal of Agricultural and Food Chemistry*, 51(7): 2070-2075.

Cares, M. G., Vargas, Y., Gaete, L., Sainz, J., & Alarcón, J. (2010). Ultrasonically assisted extraction of bioactive principles from *Quillaja Saponaria Molina*. *Physics Procedia*, 3(1): 169-178.

Carrera, C., Ruiz-Rodríguez, A., Palma, M., & Barroso, C. G. (2015). Ultrasound-assisted extraction of amino acids from grapes. *Ultrasonics Sonochemistry*, 22: 499-505.

Carvalho, C. M., & Cabral, J. M. (2000). Reverse micelles as reaction media for lipases. *Biochimie*, 82(11): 1063-1085.

Castaldo, D., Quagliuolo, L., Servillo, L., Balestrieri, C., & Giovane, A. (1989). Isolation and characterization of pectin methylesterase from apple fruit. *Journal of Food Science*, 54(3): 653-655.

Castilho, L. R., Medronho, R. A., & Alves, T. L. (2000). Production and extraction of pectinases obtained by solid state fermentation of agroindustrial residues with *Aspergillus niger*. *Bioresource Technology*, 71(1): 45-50.

Celestino, S. M. C., de Freitas, S. M., Medrano, F. J., de Sousa, M. V., & Ferreira Filho, E. X. (2006). Purification and characterization of a novel pectinase from *Acrophialophora nainiana* with emphasis on its physicochemical properties. *Journal of Biotechnology*, 123(1): 33-42.

Chakraborty, S., Khopade, A., Biao, R., Jian, W., Liu, X. Y., Mahadik, K., Balasaheb, C., Zhang, L., & Kokare, C. (2011). Characterization and stability studies on surfactant, detergent and oxidant stable α-amylase from marine haloalkaliphilic *Saccharopolyspora sp*. A9. *Journal of Molecular Catalysis B: Enzymatic*, 68(1): 52-58.

Chemat, F., Tomao, V., & Virot, M. (2008). *Ultrasound-assisted extraction in food analys*is. Handbook of Food Analysis Instruments, 85-103.

Chen, Y. L., Su, C. K., & Chiang, B. H. (2006). Optimization of reversed micellar extraction of chitosanases produced by *Bacillus cereus*. *Process Biochemistry*, 41(4): 752-758.

Chen, Y., Luo, H., Gao, A., & Zhu, M. (2011). Ultrasound-assisted extraction of polysaccharides from litchi *(Litchi chinensis Sonn)* seed by response surface methodology and their structural characteristics. *Innovative Food Science & Emerging Technologies*, 12(3): 305-309.

Chiliveri, S. R., & Linga, V. R. (2014). A novel thermostable, alkaline pectate lyase from *Bacillus tequilensis* SV11 with potential in textile industry. *Carbohydrate Polymers*, 111: 264-272.

Chitturi, S., Talatam, V. G., & Vuppu, S. (2013). Studies on protein content, protease activity, antioxidants potential, melanin composition, glucosinolate and pectin constitution with brief statistical analysis in some medicinally significant fruit peels. *Der Pharmacia Lettre Journal*, 5(1): 13-23.

Chow, Y. H., Yap, Y. J., Tan, C. P., Anuar, M. S., Tejo, B. A., Show, P. L., Ariff, A. B., Ng, E., & Ling, T. C. (2015). Characterization of bovine serum albumin partitioning behaviors in polymer-salt aqueous two-phase systems. *Journal of Bioscience and Bioengineering*, 120(1): 85-90.

Christgau, S., Kofod, L. V., Halkier, T., Andersen, L. N., Hockauf, M., Dörreich, K., Dalbøge, H., & Kauppinen, S. (1996). Pectin methyl esterase from *Aspergillus aculeatus*: expression cloning in yeast and characterization of the recombinant enzyme. *The Biochemical Journal*, 319: 705-712.

Ciardiello, M. A., Tamburrini, M., Tuppo, L., Carratore, V., Giovane, A., Mattei, B., & Camardella, L. (2004). Pectin methylesterase from kiwi and kaki fruits: purification, characterization, and role of pH in the enzyme regulation and interaction with the kiwi proteinaceous inhibitor. *Journal of Agricultural and Food Chemistry*, 52(25): 7700-7703.

Ciardiello, M. A., Tamburrini, M., Tuppo, L., Carratore, V., Giovane, A., Mattei, B., & Camardella, L. (2004). Pectin methylesterase from kiwi and kaki fruits: purification, characterization, and role of pH in the enzyme regulation and interaction with the kiwi proteinaceous inhibitor. *Journal of Agricultural and Food Chemistry*, 52(25): 7700-7703.

Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E., & García-Villanova, B. (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International*, 44(7): 2047-2053.

d'Alessandro, L. G., Kriaa, K., Nikov, I., & Dimitrov, K. (2012). Ultrasound assisted extraction of polyphenols from black chokeberry. *Separation and Purification Technology*, 93: 42-47.

da Cunha, R. L., Hubinger, M. D., Sato, A. C. K., Vieira, G. S. & Siddiq, M. (2012). *Guava: Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging.* New Jersey: John Wiley & Sons. Inc.

da Silva, L. H., & Meirelles, A. J. (2001). Phase equilibrium and protein partitioning in aqueous mixtures of maltodextrin with polypropylene glycol.*Carbohydrate Polymers*, 46(3): 267-274.

da Silva, N. R., Ferreira, L. A., Madeira, P. P., Teixeira, J. A., Uversky, V. N., & Zaslavsky, B. Y. (2015). Effect of sodium chloride on solute–solvent

interactions in aqueous polyethylene glycol–sodium sulfate two-phase systems. *Journal of Chromatography A*, 1425: 51-61.

Damak, N., Hadj-Taieb, N., Bonnin, E., Bacha, A. B., & Gargouri, A. (2011). Purification and biochemical characterization of a novel thermoactive fungal pectate lyase from *Penicillium occitanis*. *Process Biochemistry*, 46(4): 888-893.

Daniel, R. M., Danson, M. J., & Eisenthal, R. (2001). The temperature optima of enzymes: a new perspective on an old phenomenon. *Trends in Biochemical Sciences*, 26(4): 223-225.

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(a).

de Brito Cardoso, G., Souza, I. N., Pereira, M. M., Freire, M. G., Soares, C. M. F., & Lima, Á. S. (2014). Aqueous two-phase systems formed by biocompatible and biodegradable polysaccharides and acetonitrile.*Separation and Purification Technology*, 136: 74-80.

de Lemos, L. R., da Rocha Patrício, P., Rodrigues, G. D., de Carvalho, R. M. M., da Silva, M. C. H., & da Silva, L. H. M. (2011). Liquid–liquid equilibrium of aqueous two-phase systems composed of poly (ethylene oxide) 1500 and different electrolytes ($(NH_4)^2$ SO_4, $ZnSO_4$and K_2HPO_4): Experimental and correlation. *Fluid Phase Equilibria*, 305(1): 19-24.

Demir, N., Acar, J., Sarıoğlu, K., & Mutlu, M. (2001). The use of commercial pectinase in fruit juice industry. Part 3: Immobilized pectinase for mash treatment. *Journal of Food Engineering*, 47(4): 275-280.

Dhembare, A. J., Kakad, S. L., & Rana, R. (2015). Effect of pH, temperature and kinetics of pectinase enzyme using *Aspergillus niger* by solid-state of fermentation. *Der Pharmacia Sinica*, 2015:6(8): 1-5.

Diamond, A. D., & Hsu, J. T. (1990). Protein partitioning in PEG/dextran aqueous two-phase systems. *AIChE Journal*, 36(7): 1017-1024.

Dixit, S., upadhyay, S., Singh, H., Pandey, B., Chandrashekar, K., & Verma, P. (2013). Pectin methylesterase of Datura species, purification, and characterization from *Datura stramonium* and its application. *Plant Signaling & Behavior*, *8*(10): 1-8.

Djilani, A., Legseir, B., Soulimani, R., Dicko, A., & Younos, C. (2006). New extraction technique for alkaloids. *Journal of the Brazilian Chemical Society*, 17(3): 518-520.

Dong, X., Zhao, M., Shi, J., Yang, B., Li, J., Luo, D., Jiang, G., & Jiang, Y. (2011). Effects of combined high-pressure homogenization and enzymatic treatment on extraction yield, hydrolysis and function properties of peanut proteins.*Innovative Food Science & Emerging Technologies*, 12(4): 478-483.

Dreyer, S. E. (2008). *Aqueous two-phase extraction of proteins and enzymes using tetraalkylammonium-based ionic liquids*. PhD Thesis, Universität Rostock.

Dutra-Molino, J. V., Feitosa, V. A., de Lencastre-Novaes, L. C., Santos-Ebinuma, V. D. C., Lopes, A. M., Jozala, Marques, D. A. V., Malpeidi, L. P., A. F., & Júnior, A. P. (2014). Biomolecules extracted by ATPS: practical examples. *Revista Mexicana de Ingeniería Química*, 13(2): 359-377.

Dutra-Molino, J. V., Feitosa, V. A., Novaes, L. C. D. L., Ebinuma, V. D. C. S., Lopes, A. M., Jozala, A. F., Marques, D. V., Malpiedi, L. P., & Pessoa Júnior, A. (2014). Biomolecules extracted by ATPS: practical examples. *Revista Mexicana de Ingeniería Química*, 13(2): 359-377.

Ebringerová, A., & Hromádková, Z. (2010). An overview on the application of ultrasound in extraction, separation and purification of plant polysaccharides.*Central European Journal of Chemistry*, 8(2): 243-257.

Eipeson, W. E., & Bhowmik, S. R. (1992). Indian fruit and vegetable processing industry potential and challenges. *Indian Food Packer*, 46: 7-12.

El-Ahmady, S. H., Ashour, M. L., & Wink, M. (2013). Chemical composition and anti-inflammatory activity of the essential oils of *Psidium guajava* fruits and leaves. *Journal of Essential Oil Research*, 25(6): 475-481.

El-Batal, A. I., Osman, E. M., & Shaima, I. A. M. (2013). Optimization and characterization of polygalacturonase enzyme produced by gamma irradiated Penicillium citrinum. *Journal of Chemical and Pharmaceutcal Researchs*, 5(1): 336-347.

El-Deek, A. A., Asar, M. A., Hamdy, S. M., & Abdalla, A. A. (2009). Utilization of guava by products in broiler finisher diets. *Egyption Poultry Science Association Journal*, 29: 53-79.

Ertugay Eskin, N. A. M., & Hoehn. (2013). *Biochemistry of foods*. In: Fruits and Vegetables. London, UK: Elsevier Incorporated.

Eskin, N. A. M., & Hoehn. (2013). *Biochemistry of foods*. In: Fruits and Vegetables. London, UK: Elsevier Incorporated.

Falleh, H., Ksouri, R., Lucchessi, M. E., Abdelly, C., & Magné, C. (2012). Ultrasound-assisted extraction: Effect of extraction time and solvent power on the levels of polyphenols and antioxidant activity of *Mesembryanthemum*

edule L. Aizoaceae shoots. *Tropical Journal of Pharmaceutical Research*, 11(2): 243-249.

Fayyaz, A., Asbi, B. A., Ghazali, H. M., Man, Y. C., & Jinap, S. (1995). Kinetics of papaya pectinesterase. *Food Chemistry*, 53(2): 129-135.

Ferreira, A. M., Coutinho, J. A., Fernandes, A. M., & Freire, M. G. (2014). Complete removal of textile dyes from aqueous media using ionic-liquid-based aqueous two-phase systems. *Separation and Purification Technology*, 128: 58-66.

Food and Agriculture Organization (2010). Tropical fruits. Available from: http://www.fao.org/docrep/006/y5143e/y5143e1a.htm. Accessed 18.3.16

Freire, M. G., Louros, C. L., Rebelo, L. P. N., & Coutinho, J. A. (2011). Aqueous biphasic systems composed of a water-stable ionic liquid+ carbohydrates and their applications. *Green Chemistry*, 13(6): 1536-1545.

Gaffe, J., Tiznado, M. E., & Handa, A. K. (1997). Characterization and functional expression of a ubiquitously expressed tomato pectin methylesterase. *Plant Physiology*, 114(4): 1547-1556.

Gautam, S., Mukherjee, J., Roy, I., & Gupta, M. N. (2012). Emerging trends in designing short and efficient protein purification protocols. *American Journal of Biochemistry and Biotechnology*, 8(4): 230-254.

Ghafoor, K., Choi, Y. H., Jeon, J. Y., & Jo, I. H. (2009). Optimization of ultrasound-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from grape *(Vitis vinifera)* seeds. *Journal of Agricultural and Food Chemistry*, 57(11):4988-4994.

Gogate, P. R. (2008). Cavitational reactors for process intensification of chemical processing applications: a critical review. *Chemical Engineering and Processing: Process Intensification*, 47(4): 515-527.

Goja, A. M., Yang, H., Cui, M., & Li, C. (2013). Aqueous two-phase extraction advances for bioseparation. *Journal of Bioprocessing & Biotechniques*, 4(140): 1-8.

Gonzalez, S. L., & Rosso, N. D. (2011). Determination of pectin methylesterase activity in commercial pectinases and study of the inactivation kinetics through two potentiometric procedures. *Food Science and Technology (Campinas)*, 31(2): 412-417.

Grabski, A. C. (2009). Advances in preparation of biological extracts for protein purification. *Methods in Enzymology*, 463: 285-303.

Gummadi, S. N., & Kumar, D. S. (2006). Enhanced production of pectin lyase and pectate lyase by *Debaryomyces nepalensis* in submerged fermentation by statistical methods. *American Journal of Food Technology*,*1*(1): 19-33.

Gummadi, S. N., & Kumar, D. S. (2008). Batch and fed batch production of pectin lyase and pectate lyase by novel strain *Debaryomyces nepalensis* in bioreactor. *Bioresource Technology*, 99(4): 874-881.

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., Bradoo, S., & Saxena, R. K. (1999). Aqueous two-phase systems: An attractive technology for downstream processing of. *Current Science*, 77(4): 520-523.

Gutowski, K. E., Broker, G. A., Willauer, H. D., Huddleston, J. G., Swatloski, R. P., Holbrey, J. D., & Rogers, R. D. (2003). Controlling the aqueous miscibility of ionic liquids: aqueous biphasic systems of water-miscible ionic liquids and water-structuring salts for recycle, metathesis, and separations.*Journal of the American Chemical Society*, 125(22): 6632-6633.

Hagarová, D., & Breier, A. (1995). Distribution of proteins in aqueous two-phase systems formed by dextran and polyethylene glycol. Influence of protein hydrophobicity. *General Physiology and Biophysics*, 14: 277-292.

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

Hayashida, K., Kunimoto, K., Shiraishi, F., Kawakami, K., & Arai, Y. (1990). Enzymatic hydrolysis of soluble starch in a polyethylene glycol-dextran aqueous two-phase system. *Journal of Fermentation and Bioengineering*, 69(4): 240-243.

Headon, D. R., & Walsh, G. (1994). The industrial production of enzymes. *Biotechnology Advances*, 12(4): 635-646.

Hedhammar, M., Karlström, A. E., & Hober, S. (2006). Chromatographic methods for protein purification. In Royal Institute of Technology, A. U. C., Dept. of Biotechnology, SE-10691 Stockholm, Sweden, Ed.

Hemavathi, A. B., & Raghavarao, K. S. M. S. (2011). Differential partitioning of β-galactosidase and β-glucosidase using aqueous two phase extraction.*Process Biochemistry*, 46(3): 649-655.

Herodež, Š. S., Hadolin, M., Škerget, M., & Knez, Ž. (2003). Solvent extraction study of antioxidants from Balm *(Melissa officinalis L.)* leaves. *Food Chemistry*, 80(2): 275-282.

Herrera, M. C., & De Castro, M. L. (2005). Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. *Journal of Chromatography A*, 1100(1): 1-7.

Ho, R., Violette, A., Cressend, D., Raharivelomanana, P., Carrupt, P. A., & Hostettmann, K. (2012). Antioxidant potential and radical-scavenging effects of flavonoids from the leaves of *Psidium cattleianum* grown in French Polynesia. *Natural Product Research*, 26(3): 274-277.

Hong, J., Ye, X., Wang, Y., & Zhang, Y. H. P. (2008). Bioseparation of recombinant cellulose-binding module-proteins by affinity adsorption on an ultra-high-capacity cellulosic adsorbent. *Analytica chimica acta*, 621(2): 193-199.

Hoondal, G., Tiwari, R., Tewari, R., Dahiya, N. B. Q. K., & Beg, Q. (2002). Microbial alkaline pectinases and their industrial applications: a review. *Applied Microbiology and Biotechnology*, 59(4-5): 409-418.

Hoondal, G., Tiwari, R., Tewari, R., Dahiya, N. B. Q. K., & Beg, Q. (2002). Microbial alkaline pectinases and their industrial applications: a review. *Applied Microbiology and Biotechnology*, 59(4-5): 409-418.

Hoondal, G., Tiwari, R., Tewari, R., Dahiya, N. B. Q. K., & Beg, Q. (2002). Microbial alkaline pectinases and their industrial applications: a review. *Applied Microbiology and Biotechnology*, 59(4-5): 409-418.

Ibañez, E., Herrero, M., Mendiola, J. A., & Castro-Puyana, M. (2012*). Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates.*In Marine Bioactive Compound. Hayes, M. (Ed.) US: Springer Science and Business Media, 55-98.

Ibarra-Herrera, C. C., Aguilar, O., & Rito-Palomares, M. (2011). Application of an aqueous two-phase systems strategy for the potential recovery of a recombinant protein from alfalfa *(Medicago sativa). Separation and Purification Technology*, 77(1): 94-98.

Ibrahim, D., Salikin, N. H., LimSheh, H., Ahmad, R., & Weloosamy, H. (2013). Pomelo peels as alternative substrate for extracellular pectinase production by *Aspergillus niger* HFM-8. *Malaysian Journal of Microbiology*, 9(4): 308-316.

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

Illanes, A. (Ed.). (2008). *Enzyme biocatalysis: principles and applications*. New York: Springer Science & Business Media.

Imsabai, W., Ketsa, S., & van Doorn, W. G. (2002). Effect of temperature on softening and the activities of polygalacturonase and pectinesterase in durian fruit. *Postharvest Biology and Technology*, 26(3): 347-351.

Iqbal, M., Tao, Y., Xie, S., Zhu, Y., Chen, D., Wang, X., Huang, L., Peng, D., Sattar, A., Shabbir, M.A.B., & Hussain, H.I. (2016). Aqueous two-phase system (ATPS): an overview and advances in its applications. *Biological Procedures Online*, 18(1):8.

Irshad, M., Anwar, Z., Mahmood, Z., Aqil, T., Mehmmod, S., & Nawaz, H. (2014). Bio-processing of agro-industrial waste orange peel for induced production of pectinase by *Trichoderma viridi;* its purification and characterization. *Turkish Journal of Biochemistry*, 39(1): 9-18.

Janson, J. C. & Jönsson, J. A. (2012). *Introduction to chromatography*. In: Protein purification: principles, high resolution methods, and applications. Janson, J. C. (Ed.). USA: John Wiley & Sons.

Jaramillo, P. M. D., Gomes, H. A. R., de Siqueira, F. G., Homem-de-Mello, M., Ferreira Filho, E. X., & Magalhães, P. O. (2013). Liquid–liquid extraction of pectinase produced by *Aspergillus oryzae* using aqueous two-phase micellar system. *Separation and Purification Technology*, 120: 452-457.

Jauneau, A., Morvan, C., Fenyo, J. C., & Demarty, M. (1988). Pectate lyase production by *Bacillus subtilis* in a membrane bioreactor. *Applied Microbiology and Biotechnology*, *29*(2-3): 234-238.

Jayani, R. S., Saxena, S., & Gupta, R. (2005). Microbial pectinolytic enzymes: a review. *Process Biochemistry*, 40(9): 2931-2944.

Jiang, H., Dian, W., & Wu, P. (2003). Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. *Phytochemistry*, 63(1): 53-59.

Jiménez-Escrig, A., Rincón, M., Pulido, R., & Saura-Calixto, F. (2001). Guava fruit (Psidium guajava L.) as a new source of antioxidant dietary fiber.*Journal of Agricultural and Food Chemistry*, 49(11): 5489-5493.

Jiménez-Escrig, A., Rincón, M., Pulido, R., & Saura-Calixto, F. (2001). Guava fruit (Psidium guajava L.) as a new source of antioxidant dietary fiber.*Journal of Agricultural and Food Chemistry*, 49(11): 5489-5493.

Johansson, G. (1985). Aqueous two-phase systems in protein purification. *Journal of Biotechnology*, 3(1-2): 11-18.

Johansson, H. O., Feitosa, E., & Junior, A. P. (2011). Phase diagrams of the aqueous two-phase systems of poly(ethyleneglycol)/sodium polyacrylate /salts. *Polymers*, 3(1): 587-601.

Joseph, B., & Priya, M. (2011). Review on nutritional, medicinal and pharmacological properties of guava *(Psidium guajava Linn.). International Journal of Pharma and Bio Science*, 2(1): 53-69.

Joshi, V. K., Parmar, M., & Rana, N. (2011). Purification and characterization of pectinase produced from Apple pomace and evaluation of its efficacy in fruit juice extraction and clarification. *Indian Journal of Natural Products and Resources*, 2(2): 189-197.

Jurick, W. M., Vico, I., McEvoy, J. L., Whitaker, B. D., Janisiewicz, W., & Conway, W. S. (2009). Isolation, purification, and characterization of a polygalacturonase produced in *Penicillium solitum*-decayed 'Golden Delicious' apple fruit. *Phytopathology*, 99(6): 636-641.

Kalipatnapu, S., & Chattopadhyay, A. (2005). Membrane protein solubilization: recent advances and challenges in solubilization of serotonin1A receptors. *IUBMB Life*, 57(7): 505-512.

Kant, S., & Gupta, R. (2012). Purification of pectin methylesterase from *Lycopersicon esculentum* and its application. *Protein and Peptide Letters*, 19(11): 1205-1211.

Kapoor, M., & Kuhad, R. C. (2002). Improved polygalacturonase production from Bacillus sp. MG-cp-2 under submerged (SmF) and solid state (SSF) fermentation. *Letters in Applied Microbiology*, 34(5): 317-322.

Kar, S.,& Ray, R. C. (2008). Partial characterization of extracellular thermostable Ca^{2+} inhibited α–amylase prodcution by *Streptomyces erunpens* MTCC 7317. *Journal of Scientific and Industrial Research*, 67: 58-64.

Karakuş, E., & Pekyardımcı, Ş. (2009). Immobilization of apricot pectinesterase (Prunusarmeniaca L.) on porous glass beads and its characterization. *Journal of Molecular Catalysis B: Enzymatic*, 56(1): 13-19.

Karbalaei-Heidari, H. R., & Rastegari, B. (2014). Isolation and Partial Characterization of a Bacterial Thermostable Polymethyl Galacturonase from a Newly Isolated *Bacillus sp*. strain BR1390. *Iranian Journal of Biotechnology*, 12(4): 41-46.

Karimi, R., Kazzazi, M., & Vatanprast, M. (2015). Biochemical characterization of pectinase activity from the digestive midgut fluid of larvae and adult of the Colorado potato beetle, *Leptinotarsa decemlineata* (Col: Chrysomelidae). *Arthropods*, 4(1): 22-31.

Kashyap, D. R., Vohra, P. K., Chopra, S., & Tewari, R. (2001). Applications of pectinases in the commercial sector: a review. *Bioresource Technology*, 77(3): 215-227.

Kaur, G., Kumar, S., & Satyanarayana, T. (2004). Production, characterization and application of a thermostable polygalacturonase of a thermophilic mould *Sporotrichum* thermophile *Apinis*. *Bioresource Technology*, 94(3): 239-243.

Keharom, S., Mahachai, R., & Chanthai, S. (2016). The optimization study of α-amylase activity based on central composite design-response surface

methodology by dinitrosalicylic acid method. *International Food Research Journal*, 23(1): 10-17.

Ketnawa, S., Rungraeng, N., & Rawdkuen, S. (2017). Phase partitioning for enzyme separation: An overview and recent applications: A mini review. *International Food Research Journal*, 24(1): 1-24.

Khan, I. G., & Barate, D. L. (2016). Effect of various parameters on activity of pectinase enzyme. *International Journal*, 4(1): 853-862.

Khan, M. K., Abert-Vian, M., Fabiano-Tixier, A. S., Dangles, O., & Chemat, F. (2010). Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis L.)* peel. *Food Chemistry*, 119(2): 851-858.

Khan, S. H., Butt, M. S., Sharif, M. K., Sameen, A., Mumtaz, S., & Sultan, M. T. (2011). Functional properties of protein isolates extracted from stabilized rice bran by microwave, dry heat, and parboiling. *Journal of Agricultural and Food Chemistry*, 59(6): 2416-2420.

Khatri, B. P., Bhattarai, T., Shrestha, S., & Maharjan, J. (2015). Alkaline thermostable pectinase enzyme from *Aspergillus niger strain* MCAS2 isolated from Manaslu Conservation Area, Gorkha, Nepal. *SpringerPlus*, 1(4): 1-8.

Khoramnia, A., Lai, O. M., Ebrahimpour, A., Tanduba, C. J., Voon, T. S., & Mukhlis, S. (2010). Thermostable lipase from a newly isolated *Staphylococcus xylosus* strain; process optimization and characterization using RSM and ANN. *Electronic Journal of Biotechnology*, 13(5): 15-16.

Killion, K. H. (2000). The Review of Natural Products. *Facts and Comparison, USA*, 250–251.

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.

Kim, J. W., Nagaoka, T., Ishida, Y., Hasegawa, T., Kitagawa, K., & Lee, S. C. (2009). Subcritical water extraction of nutraceutical compounds from citrus pomaces. *Separation Science and Technology*, 44(11): 2598-2608.

Kim, J., Shiu, S. H., Thoma, S., Li, W. H., & Patterson, S. E. (2006). Patterns of expansion and expression divergence in the plant polygalacturonase gene family. *Genome Biology*, *7*, R87.

Kim, S. M., & Zayas, J. F. (1989). Processing parameter of chymosin extraction by ultrasound. *Journal of Food Science*, 54: 700.

Klug-Santner, B. G., Schnitzhofer, W., Vršanská, M., Weber, J., Agrawal, P. B., Nierstrasz, V. A., & Guebitz, G. M. (2006). Purification and characterization of a new bioscouring pectate lyase from *Bacillus pumilus* BK2. *Journal of Biotechnology*, 121(3), 390-401.

Kluskens, L. D., Van Alebeek, G. J. W., & Voragen, A. G. (2003). Molecular and biochemical characterization of the thermoactive family 1 pectate lyase from the hyperthermophilic bacterium *Thermotoga maritima*. *Biochemical Journal*, 370(2): 651-659.

Kobayashi, T., Koike, K., Yoshimatsu, T., Higaki, N., Suzumatsu, A., Ozawa, T., Hatada, Y., & Ito, S. (1999). Purification and properties of a low-molecular-weight, high-alkaline pectate lyase from an alkaliphilic strain of *Bacillus*. *Bioscience, Biotechnology, and Biochemistry*, 63(1): 65-72.

Kobori, C. N., & Jorge, N. (2005). Caracterização dos óleos de algumas sementes de frutas como aproveitamento de resíduos industriais. *Ciência e agrotecnologia*, 29(5): 1008-1014.

Kohli, P., Kalia, M., & Gupta, R. (2015). Pectin methylesterases: A review. *Journal of Bioprocessing & Biotechniques*, 5(5): 1-7.

Kolhe, P., Amend, E., & K Singh, S. (2010). Impact of freezing of buffered solutions and consequences for monoclonal antibody aggregation. *Biotechnology Progress*, 26(3): 727-733.

Kollar, A. (1998). Characterization of an endo-polygalacturonase produced by the apple scab fungus, *Venturia inaequalis*. *Mycological Research*, *102*(3), 313-319.

Kopperschläger, G., & Birkenmeier, G. (1993). *Affinity partitioning of biomolecules in aqueous two-phase system. In: Molecular interactions in bioseparation*. Ngo, T. T. (Ed.), New York: Springer Science and Business Media, 499-510.

Koser, S., Anwar, Z., Iqbal, Z., Anjum, A., Aqil, T., Mehmood, S., & Irshad, M. (2014). Utilization of *Aspergillus oryzae* to produce pectin lyase from various agro-industrial residues. *Journal of Radiation Research and Applied Sciences*, *7*(3): 327-332.

Kuhad, R. C., Kapoor, M., & Rustagi, R. (2004). Enhanced production of an alkaline pectinase from Streptomyces sp. RCK-SC by whole-cell immobilization and solid-state cultivation. *World Journal of Microbiology and Biotechnology*, 20(3): 257-263.

Kulaguin-Chicaroux, A., & Zeiner, T. (2014). Novel aqueous two-phase system based on a hyperbranched polymer. *Fluid Phase Equilibria*, 362: 1-10.

Kulkarni, V. M., & Rathod, V. K. (2014). Mapping of an ultrasonic bath for ultrasound assisted extraction of mangiferin from *Mangifera indica* leaves.*Ultrasonics Sonochemistry*, 21(2): 606-611.

Kumar, P., & Sharma, S. M. (2015). An overview of purification methods for proteins. *International Journal of Applied Research*, 1(12): 450-459.

Kumar, Y. S., Kumar, P. V., & Reddy, O. V. S. (2012). Pectinase production from mango peel using *Aspergillus foetidus* and its application in processing of mango juice. *Food Biotechnology*, 26(2): 107-123.

Kwee, L. T. and Chong, K. K. (1990). *Guava in Malaysia: Production, pest and diseases*. Kuala Lumpur, Malaysia: Tropical Press Sdn. Bhd.

Laemmli, U.K. (1970) Nature (London) 227, 680–685.

Lara-Márquez, A., Zavala-Páramo, M. G., López-Romero, E., & Camacho, H. C. (2011). Biotechnological potential of pectinolytic complexes of fungi. *Biotechnology Letters*, 33(5): 859-868.

Lee, J., Ashokkumar, M., Kentish, S., & Grieser, F. (2005). Determination of the size distribution of sonoluminescence bubbles in a pulsed acoustic field.*Journal of the American Chemical Society*, 127(48): 16810-16811.

Lee, S. Y., Khoiroh, I., Ling, T. C., & Show, P. L. (2017). Enhanced recovery of lipase derived from *Burkholderia cepacia* from fermentation broth using recyclable ionic liquid/polymer-based aqueous two-phase systems. *Separation and Purification Technology*, 179: 152-160.

Lee, S., Choi, H. K., Cho, S. K., & Kim, Y. S. (2010). Metabolic analysis of guava (Psidium guajava L.) fruits at different ripening stages using different data-processing approaches. *Journal of Chromatography B*, 878(29): 2983-2988.

Lee, S., Choi, H. K., Cho, S. K., & Kim, Y. S. (2010). Metabolic analysis of guava (Psidium guajava L.) fruits at different ripening stages using different data-processing approaches. *Journal of Chromatography B*, 878(29): 2983-2988.

Lee, W. C., Yusof, S., Hamid, N. S. A., & Baharin, B. S. (2006). Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). *Journal of Food Engineering*, 73(1): 55-63.

Leong, Y. K., Show, P. L., Lan, J. C. W., & Loh, H. S. (2015). Thermoseparating Aqueous Two-Phase System: Recent Trends and Applications. *Chemical Engineering*, 45(2015): 1249- 1254.

Leuko, S., Goh, F., Ibáñez-Peral, R., Burns, B. P., Walter, M. R., & Neilan, B. A. (2008). Lysis efficiency of standard DNA extraction methods for *Halococcus spp.* in an organic rich environment. *Extremophiles*, 12(2): 301-308.

Li, F., Li, S., Li, H. B., Deng, G. F., Ling, W. H., Wu, S., Xu, X., & Chen, F. (2013). Antiproliferative activity of peels, pulps and seeds of 61 fruits. *Journal of Functional Foods*, 5(3): 1298-1309.

Li, H. (2002). *Ultrasound and microwave assisted extraction of soybean oil*. Master Thesis, University of Tennessee, Knoxville Trace: Tennessee Research and Creative Exchange.

Li, H., Pordesimo, L., & Weiss, J. (2004). High intensity ultrasound-assisted extraction of oil from soybeans. *Food Research International*, 37(7): 731-738.

Li, J., Jiang, Z., Wu, H., Long, L., Jiang, Y., & Zhang, L. (2009). Improving the recycling and storage stability of enzyme by encapsulation in mesoporous $CaCO^3$–alginate composite gel. *Composites Science and Technology*, 69(3): 539-544.

Li, S., Yang, X., Yang, S., Zhu, M., & Wang, X. (2012). Technology prospecting on enzymes: application, marketing and engineering. *Computational and Structural Biotechnology Journal*, *2*(3), 1-11.

Li, S., Yang, X., Yang, S., Zhu, M., & Wang, X. (2012). Technology prospecting on enzymes: application, marketing and engineering. *Computational and Structural Biotechnology Journal*, *2*(3), 1-11.

Lima, Á. S., Alegre, R. M., & Meirelles, A. J. (2002). Partitioning of pectinolytic enzymes in polyethylene glycol/potassium phosphate aqueous two-phase systems. *Carbohydrate Polymers*, 50(1): 63-68.

Lin, Y. K., Ooi, C. W., Tan, J. S., Show, P. L., Ariff, A., & Ling, T. C. (2013). Recovery of human interferon alpha-2b from recombinant *Escherichia coli* using alcohol/salt-based aqueous two-phase systems. *Separation and Purification Technology*, 120: 362-366.

Liu, Q. M., Yang, X. M., Zhang, L., & Majetich, G. (2010). Optimization of ultrasonic-assisted extraction of chlorogenic acid from *Folium eucommiae* and evaluation of its antioxidant activity. *Journal of Medicinal Plants Research*, 4(23): 2503-2511.

Liu, Y., Han, J., Wang, Y., Lu, Y., Zhang, G., Sheng, C., & Yan, Y. (2013). Selective separation of flavones and sugars from honeysuckle by alcohol/salt aqueous two-phase system and optimization of extraction process. *Separation and Purification Technology*, 118: 776-783.

Liu, Y., Wu, Z., Zhang, Y., & Yuan, H. (2012). Partitioning of biomolecules in aqueous two-phase systems of polyethylene glycol and non-ionic surfactant. *Biochemical Engineering Journal*, 69: 93-99.

Liza, M. S., Rahman, R. A., Mandana, B., Jinap, S., Rahmat, A., Zaidul, I. S. M., & Hamid, A. (2010). Supercritical carbon dioxide extraction of bioactive flavonoid from *Strobilanthes crispus* (Pecah Kaca). *Food and Bioproducts Processing*, 88(2): 319-326.

Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J. (2000). *Purifying, detecting, and characterizing proteins*, In: Molecular cell biology, New York: W. H. Freeman Company.

Lu, T., Li, Z., Huang, J., & Fu, H. (2008). Aqueous surfactant two-phase systems in a mixture of cationic gemini and anionic surfactants. *Langmuir*, 24(19): 10723-10728.

Ma, G. H., Wang, P., & Su, Z. G. (2009). Nanoscience and enzyme. *Journal of Chinese Basic Science*, 5(1): 49–54.

Mađarev, S., Antov, M., & Peričin, D. (2004). Partitioning of pectinases in aqueous two-phase system polyethylene glycol/dextran 500,000. Proceedings of the 6th International*: Symposium "Young People and Multidisciplinary Research.* Romania, Temisoara.

Mađarev, S., Antov, M., & Peričin, D. (2006). Partitioning of pectinases in aqueous two-phase system polyethylene glycol/dextran 500,000. *Analysis of the Faculty of Engineering Hunedoara*, 4(3): 181-185.

Makris, D. P., Boskou, G., & Andrikopoulos, N. K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis*, 20(2): 125-132.

Mandal, S. C., Mandal, V., & Das, A. K. (2015). *Essentials of botanical extraction: principles and applications*. US: Academic Press.

Marina, Z., & Noriham, A. (2014). Quantification of total phenolic compound and in vitro antioxidant potential of fruit peel extracts. *International Food Research Journal*, 21(5): 1925-1929.

Marín-Rodríguez, M. C. (2001). *Investigation of the role of pectate lyase in banana fruit softening*, PhD Thesis, University of Greenwich.

Marques, C. F., Mourão, T., Neves, C. M., Lima, Á. S., Boal-Palheiros, I., Coutinho, J. A., & Freire, M. G. (2013). Aqueous biphasic systems composed of ionic liquids and sodium carbonate as enhanced routes for the extraction of tetracycline. *Biotechnology Progress*, 29(3): 645-654.

Martin, F. W. (1984). *Handbook of Tropical Food Crops*. Florida: CRC Press, 254-256.

Martínez-Maqueda, D., Hernández-Ledesma, B., Amigo, L., Miralles, B., & Gómez-Ruiz, J. Á. (2013). *Extraction/fractionation techniques for proteins and peptides and protein digestion*. In *Proteomics in foods:Principles and applications*. New York: Springer Science and Business Media., 21-50.

Martins, E. D. S., Leite, R. S. R., da Silva, R., & Gomes, E. (2013). Purification and properties of polygalacturonase produced by thermophilic fungus *Thermoascus aurantiacus* CBMAI-756 on solid-state fermentation. *Enzyme Research*, 2013: 1-7.

Martins, E. S., Silva, D., Da Silva, R., & Gomes, E. (2002). Solid state production of thermostable pectinases from thermophilic *Thermoascus aurantiacus*. *Process Biochemistry*, 37(9): 949-954.

Medina-Escobar, N., Cárdenas, J., Moyano, E., Caballero, J. L., & Muñoz-Blanco, J. (1997). Cloning, molecular characterization and expression pattern of a strawberry ripening-specific cDNA with sequence homology to pectate lyase from higher plants. *Plant Molecular Biology*, 34(6): 867-877.

Mehrnoush, A., Mustafa, S., Sarker, M. Z. I., & Yazid, A. M. M. (2012). Optimization of serine protease purification from mango (Mangifera indica cv. Chokanan) peel in polyethylene glycol/dextran aqueous two phase system. *International Journal of Molecular Sciences*, 13(3): 3636-3649.

Mei, Y., Chen, Y., Zhai, R., & Liu, Y. (2013). Cloning, purification and biochemical properties of a thermostable pectinase from *Bacillus halodurans* M29. *Journal of Molecular Catalysis B: Enzymatic*, 94: 77-81.

Merchuk, J. C., Andrews, B. A., & Asenjo, J. A. (1998). Aqueous two-phase systems for protein separation: studies on phase inversion. *Journal of Chromatography B: Biomedical Sciences and Applications*, *711*(1), 285-293.

Middelberg, A. P. (2000). *Microbial cell disruption by high-pressure homogenization*. In: Downstream Processing of Proteins: Methods and Protocols. Desai, M. A. (Ed.), New Jersey: Humana Press, 11-21.

Miller, G. L. (1959). Determination of reducing sugar by DNS method. *Analytical Chemistry*, 31: 426-428.

Miranda, M. V., Fernandez-Lahore, H. M., Dobrecky, J., and Cascone, O. (1998). The extractive purification of peroxidase from plant raw materials in aqueous two-phase systems. *Acta Biotechnologica*, 18: 179-188.

Mirhosseini, H., Tan, C. P., Hamid, N. S., & Yusof, S. (2008). Effect of Arabic gum, xanthan gum and orange oil contents on ζ-potential, conductivity, stability, size index and pH of orange beverage emulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 315(1): 47-56.

Mohamadi, H. S., Omidinia, E., & Dinarvand, R. (2007). Evaluation of recombinant phenylalanine dehydrogenase behavior in aqueous two-phase partitioning. *Process Biochemistry*, *42*(9), 1296-1301.

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.

Mohan, C. (1999). *Buffers. A Guide for the Preparation and use of Buffers in Biological Systems*. US, California:Calbiochem-Novabiochem Corporation, La Jolla.

Montgomery, D. C. (2005). Design and Analysis of Experiments: Response surface method and designs. New Jersey: John Wiley and Sons, Inc.

Montgomery, D. C. (2009). *Design and analysis of experiments*. New York: John Wiley & Sons Inc.

Morton, J. F. (1987). Guava: *Fruits of warm climates*. Miami, 356-363.

Moulton, K. J., & Wang, L. C. (1982). A Pilot-Plant Study of Continuous Ultrasonic Extraction of Soybean Protein. *Journal of Food Science*, 47(4): 1127-1129.

Murray, R. K., Granner, D. K., Mayes, P. A., & Rodwell, V. W. (2000). Harpers Biochemistry. US (Conneticut): Appleton and Lange.

Mutalib, F. A. A., Jahim, J. M., Bakar, F. D. A., Mohammad, A. W., & Hassan, O. (2014). Characterisation of new aqueous two-phase systems comprising of Dehypon® LS54 and K4484® Dextrin for potential cutinase recovery. *Separation and Purification Technology*, 123: 183-189.

Nagai, M., Katsuragi, T., Terashita, T., Yoshikawa, K., & SAKAI, T. (2000). Purification and characterization of an endo-polygalacturonase from *Aspergillus awamori*. *Bioscience, Biotechnology, and Biochemistry*, 64(8): 1729-1732.

Naganagouda, K., & Mulimani, V. H. (2008). Aqueous two-phase extraction (ATPE): an attractive and economically viable technology for downstream processing of *Aspergillus oryzae* α-galactosidase. *Process Biochemistry*, 43(11): 1293-1299.

Ng, H. S., Ooi, C. W., Mokhtar, M. N., Show, P. L., Ariff, A., Tan, J. S., Ng, E., & Ling, T. C. (2013). Extractive bioconversion of cyclodextrins by *Bacillus cereus* cyclodextrin glycosyltransferase in aqueous two-phase system. *Bioresource Technology*, 142: 723-726.

Ng, H. S., Tan, C. P., Mokhtar, M. N., Ibrahim, S., Ariff, A., Ooi, C. W., & Ling, T. C. (2012). Recovery of Bacillus cereus cyclodextrin glycosyltransferase and recycling of phase components in an aqueous two-phase system using thermo-separating polymer. *Separation and Purification Technology*, 89: 9-15.
Ng, H. S., Tan, C. P., Mokhtar, M. N., Ibrahim, S., Ariff, A., Ooi, C. W., & Ling, T. C. (2012). Recovery of Bacillus cereus cyclodextrin glycosyltransferase and recycling of phase components in an aqueous two-phase system using thermo-separating polymer. *Separation and Purification Technology*, 89: 9-15.

Ngo, L. T. A., Pham, T. L., & Le, V. V. M. (2008). Purification of Endopolygalacturonase from submerged culture of Aspergillus awamori L1 using a two-step procedure: Enzyme precipitation and gel filtration.*International Food Research Journal*, 15(2): 135-140.

Nielsen, J. E., & Christensen, T. M. (2002). Distribution of pectin methyl esterase and acetylesterase in the genus Citrus visualized by tissue prints and chromatography. *Plant Science*, 162(5): 799-807.

Nitsawang, S., Hatti-Kaul, R., & Kanasawud, P. (2006). Purification of papain from Carica papaya latex: aqueous two-phase extraction versus two-step salt precipitation. *Enzyme and Microbial Technology*, 39(5): 1103-1107.

Nogata, Y., Ohta, H., & Voragen, A. G. J. (1993). Polygalacturonase in strawberry fruit. *Phytochemistry*, 34(3): 617-620.

Noorbatcha, I. A., Ismail, N. I., & Salleh, H. M. (2011). Computer Aided Design of Polygalacturonase II from *Aspergillus niger*. *IIUM Engineering Journal*, *12*(4): 189-196.

Nunan, K. J., Davies, C., Robinson, S. P., & Fincher, G. B. (2001). Expression patterns of cell wall-modifying enzymes during grape berry development. *Planta*, 214(2): 257-264.

Nur'Alia, A. R., Siti Mazlina, M. K., & Taip, F. S. (2010). Impact of commercial pectolytic enzymes on selected properties of white dragon fruit juice. *The Instituition of Engineers*, Malaysia. 71(4): 25-31.

Nwokoro, O., & Eze, I. C. (2016). Production of polygalacturonase by *Aspergillus niger* BC23 isolated from Irvingia gabonensis (African mango) fruit. *Chemical Industry/Hemijska Industrija*, 70(6): 717-724.

Oancea, S., Grosu, C., Ketney, O., & Stoia, M. (2013). Conventional and ultrasound-assisted extraction of anthocyanins from blackberry and sweet cherry cultivars. *Acta Chimica Slovenica*, 60(2): 383-389.

Oliveira, R. D. Q., Junior, V., Lima, G., Uetanabaro, A. P. T., Koblitz, M. G. B., Góes-Neto, A., Rosa, C. A., & Assis, S. A. D. (2012). Influence of carbon source, pH, and temperature on the polygalacturonase activity of *Kluyveromyces marxianus* CCMB 322. *Food Science and Technology (Campinas)*, 32(3): 499-505.

Oliveira, R., Rodrigues, M. F., & Bernardo-Gil, M. G. (2002). Characterization and supercritical carbon dioxide extraction of walnut oil. *Journal of the American Oil Chemists' Society*, 79(3): 225-230.

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.

Oslen, H.S. (2000). Enzymes at work- A concise guide to industrial enzymes and their use. Novozymes A/S Bagsvaerd, Denmark.

Palomares, L. A., Estrada-Moncada, S., & Ramírez, O. T. (2004). *Production of recombinant proteins: Challenges and solutio*ns. In: *Recombinant Gene Expression: Reviews and Protocols*. Balbás, P., & Lorence, A. (Eds.). New Jersey: Humana Press Inc., 15-51.

Park, S. H., Min, S. G., Jo, Y. J., & Chun, J. Y. (2015). Effect of high pressure homogenization on the physicochemical properties of natural plant-based model emulsion applicable for dairy products. *Korean Journal for Food Science of Animal Resources*, 35(5): 630-637.

Pasha, K. M., Anuradha, P., & Subbarao, D. (2013). Applications of pectinases in industrial sector. *International Journal of Pure and Applied Sciences and Technology*, 16(1): 89.

Pashkoulov, D., Giannetti, I., Benvenuto, E., & De Martinis, D. (2002). Biochemical characterization of polygalacturonases from five different isolates of *Botrytis cinerea*. *Mycological Research*, 106(07): 827-831.

Pashova, S., Slokoska, L., Sheremetska, P., Krumova, E., Vasileva, L., & Angelova, M. (1999). Physiological aspects of immobilised *Aspergillus niger* cells producing polymethylgalacturonase. *Process Biochemistry*, 35(1), 15-19.

Patel, R. K., Maiti, C. S., Deka, B. C., Deshmukh, N. A., Verma, V. K., & Nath, A. (2015). Physical and biochemical changes in guava (*Psidium Guajava L*.) during various stages of fruit growth and development. *International Journal of Agriculture, Environment and Biotechnology*, 8(1), 75.

Pathak, N., Mishra, S., & Sanwal, G. G. (2000). Purification and characterization of polygalacturonase from banana fruit. *Phytochemistry*, 54(2): 147-152.

Patil, V., Chauhan, A. K., & Singh, R. P. (2014). Optimization of the spray-drying process for developing guava powder using response surface methodology. *Powder Technology*, 253: 230-236.

Paulus, A., Morhardt, C., Lehle, N., & Franzreb, M. (2014). Recovery of chymotrypsin using magnetic particles and aqueous micellar two-phase systems: influence of non-ionic surfactants on enzyme activity. *Journal of Molecular Catalysis B: Enzymatic*, 110: 165-170.

Payasi, A., Misra, P. C., & Sanwal, G. G. (2006). Purification and characterization of pectate lyase from banana *(Musa acuminata)* fruits. *Phytochemistry*, 67(9): 861-869.

Pedrolli, D. B., Monteiro, A. C., Gomes, E., & Carmona, E. C. (2009). Pectin and pectinases: production, characterization and industrial application of microbial pectinolytic enzymes. *Open Biotechnology Journal*, 3:9-18.

Pereira, J. F., Ventura, S. P., e Silva, F. A., Shahriari, S., Freire, M. G., & Coutinho, J. A. (2013). Aqueous biphasic systems composed of ionic liquids and polymers: a platform for the purification of biomolecules. *Separation and Purification Technology*, 113: 83-89.

Pereira, M., Wu, Y. T., Venâncio, A., & Teixeira, J. (2003). Aqueous two-phase extraction using thermoseparating polymer: a new system for the separation of endo-polygalacturonase. *Biochemical Engineering Journal*, 15(2): 131-138.

Pereira, M., Wu, Y. T., Venâncio, A., & Teixeira, J. (2003). Aqueous two-phase extraction using thermoseparating polymer: a new system for the separation of endo-polygalacturonase. *Biochemical Engineering Journal*, 15(2): 131-138.

Pérez-Fuentes, C., Ravanal, M. C., & Eyzaguirre, J. (2014). Heterologous expression of a Penicillium purpurogenum pectin lyase in Pichia pastoris and its characterization. *Fungal biology*, 118(5): 507-515.

Peters, T.J. (2004). Partition of cell particles and macromolecules: Separation and purification of biomolecules, cell organelles, membranes and cells in aqueous polymer two phase systems and their use in biochemical analysis and biotechnology. In: *Cell Biochemistry and Function,* Albertsson, P. A. (Ed.), 346, UK: John Wiley and Sons.

Pham, T. P. T., Cho, C. W., & Yun, Y. S. (2010). Environmental fate and toxicity of ionic liquids: A review. *Water Research*, 44: 352-372.

Prince, K., & Smith, M. (2004). Purification process scale-up. *Protein Purification Protocols*, 463-480.

Rai, P., Majumdar, G. C., Dasgupta, S. D. E. S., & De, S. (2004). Optimizing pectinase usage in pretreatment of mosambi juice for clarification by response surface methodology. *Journal of Food Engineering*, 64(3): 397-403.

Raja, S. (2013). *Aqueous two phase systems for the recovery of biomolecules from tannery wastewater*. PhD Thesis, Manipal University, Department Of Biotechnology Manipal Institute of Technology.

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: 1-11.

Raja, S., Murty, V. R., Thivaharan, V., Rajasekar, V., & Ramesh, V. (2011). Aqueous two phase systems for the recovery of biomolecules–a review.*Science and Technology*, 1(1): 7-16.

Rajendran, R., Sundaram, S. K., Radhai, R., & Rajapriya, P. (2011). Bioscouring of cotton fabrics using pectinase enzyme its optimization and comparison with

conventional scouring process. *Pakistan Journal of Biological Sciences: PJBS*, 14(9): 519-525.

Raju, E. V. N., & Divakar, G. (2013). Production of pectinase by using Bacillus circulans isolated from dump yards of vegetable wastes.*International Journal of Pharmaceutical Sciences and Research*, 4(7): 2615.

Raju, E. V. N., & Divakar, G. (2013). Production of pectinase by using Bacillus circulans isolated from dump yards of vegetable wastes. *International Journal of Pharmaceutical Sciences and Research*, 4(7): 2615.

Ramana, V. L., Regupathi, I., Rashmi, B. S., & Basavaraj, S. N. (2016). Partitioning of nitralase enzyme from pseudomonas putida in polymer/salt aqueous two phase system. In *Biotechnology and Biochemical Engineering* (pp. 93-100). Singapore: Springer Science and Business Media.

Rashad, M. M., Abdou, H. M., Shousha, W. G., Ali, M. M., & El-Sayed, N. N. (2011). Purification and characterization of the pectin lyase produced by *Pleurotus ostreatus* grown on lemon pulp waste. *Australian Journal of Basic Applied Science*, 5(8): 1377-1384.

Reid, I., & Ricard, M. (2000). Pectinase in papermaking: solving retention problems in mechanical pulps bleached with hydrogen peroxide. *Enzyme and Microbial Technology*, 26(2): 115-123.

Reis, I. A. O., dos Santos, S. B., Nascimento, L. A. S., Oliveira, N., Ventura, S. P. M., & Coutinho, J. A. P. (2011). Extraction of ascorbic acid using alcohol/phosphate potassium salt-based aqueous two phase system. In *Food process engineering in a changing world. Proceedings of the 11th International Congress on Engineering and Food, May 22-26*, 1703-1704, Athens: Cosmosware.

Rico-Castro, X., González-Amado, M., Soto, A., & Rodríguez, O. (2017). Aqueous two-phase systems with thermo-sensitive EOPO co-polymer (UCON) and sulfate salts: Effect of temperature and cation. *The Journal of Chemical Thermodynamics*.

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.

Rodrigues, G. D., de Lemos, L. R., da Silva, L. H. M., & da Silva, M. C. H. (2013). Application of hydrophobic extractant in aqueous two-phase systems for selective extraction of cobalt, nickel and cadmium. *Journal of Chromatography A*, 1279: 13-19.

Rodríguez-Fernández, D. E., Rodríguez-León, J. A., De Carvalho, J. C., Sturm, W., & Soccol, C. R. (2011). The behavior of kinetic parameters in production of

pectinase and xylanase by solid-state fermentation.*Bioresource Technology*, 102(22): 10657-10662.

Roldán, A., Palacios, V., Peñate, X., Benítez, T., & Pérez, L. (2006). Use of Trichoderma enzymatic extracts on vinification of Palomino fino grapes in the sherry region. *Journal of food engineering*, 75(3): 375-382.

Roosdiana, A., Prasetyawan, S., Mahdi, C., & Sutrisno, S. (2013). Production and characterization of *Bacillus firmus* pectinase. *The Journal of Pure and Applied Chemistry Research*, 2(1): 35-41.

Roque, A. C. A., Taipa, M. Â., & Lowe, C. R. (2005). An artificial protein L for the purification of immunoglobulins and Fab fragments by affinity chromatography. *Journal of Chromatography A*, 1064(2): 157-167.

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.

Rose, A. H. (1980). *Microbial enzymes and bioconversions* (Vol. 5). London: Academic Press Ltd.

Rostagno, M. A., Palma, M., & Barroso, C. G. (2003). Ultrasound-assisted extraction of soy isoflavones. *Journal of Chromatography A*, 1012:119–128.

Rostagno, M. A., Palma, M., & Barroso, C. G. (2004). Pressurized liquid extraction of isoflavones from soybeans. *Analytica Chimica Acta*, 522(2): 169-177.

Rothe, G. M. (2002). *Enzymes Assays after gel electrophoresis*. In: Enzyme Assays. Eisenthal, R. & Danson, M. J. (Eds.), United Kingdom: Oxford University Press.

Rouhani, S., Alizadeh, N., Salimi, S., & Haji-Ghasemi, T. (2009). Ultrasonic Assisted Extraction of Natural Pigments from Rhizomes of *Curcuma Longa L. Progress in Color, Colorants and Coatings,* 2(2009): 103-113.

Roy, I. P. S. I. T. A., & Gupta, M. N. (2002). Downstream processing of enzymes/proteins. *Proceedings- Indian National Science Academy Part B*, 68(2): 175-204.

Sakiyama, C. C. H., Paula, E. M., Pereira, P. C., Borges, A. C., & Silva, D. O. (2001). Characterization of pectin lyase produced by an endophytic strain isolated from coffee cherries. *Letters in Applied Microbiology*, 33(2): 117-121.

Salabat, A., Far, M. R., & Moghadam, S. T. (2011). Partitioning of amino acids in surfactant based aqueous two-phase systems containing the non-ionic surfactant (Triton X-100) and salts. *Journal of Solution Chemistry*, 40(1): 61-66.

Salabat, A., Moghadam, S. T., & Far, M. R. (2010). Liquid–liquid equilibria of aqueous two-phase systems composed of TritonX-100 and sodium citrate or magnesium sulfate salts. *Calphad*, 34(1): 81-83.

Salazar, D. M., Melgarejo, P., Martínez, R., Martínez, J. J., Hernández, F., & Burguera, M. (2006). Phenological stages of the guava tree *(Psidium guajava L.)*. *Scientia Horticulturae*, 108(2): 157-161.

Šalić, A., Tušek, A., Fabek, D., Rukavina, I., & Zelić, B. (2011). Aqueous two-phase extraction of polyphenols using a microchannel system–process optimization and intensification. *Food Technology and Biotechnology*, 49(4): 495-501.

Sanatan, P. T., Lomate, P. R., Giri, A. P., & Hivrale, V. K. (2013). Characterization of a chemostable serine alkaline protease from *Periplaneta americana*. *BMC biochemistry*, 14(1): 1.

Sandri, I. G., Fontana, R. C., Barfknecht, D. M., & da Silveira, M. M. (2011). Clarification of fruit juices by fungal pectinases. *LWT- Food Science and Technology*, 44(10): 2217-2222.

Sandri, I. G., Fontana, R. C., Barfknecht, D. M., & da Silveira, M. M. (2011). Clarification of fruit juices by fungal pectinases. *LWT- Food Science and Technology*, 44(10): 2217-2222.

Saoudi, B., Habbeche, A., Kerouaz, B., Haberra, S., Romdhane, Z. B., Tichati, L., Boudelaa, M., Belghith, H., Gargouri, A., & Ladjama, A. (2015). Purification and characterization of a new thermoalkaliphilic pectate lyase from *Actinomadura keratinilytica* Cpt20. *Process Biochemistry*, 50(12): 2259-2266.

Saravanan, S., Rao, J. R., Nair, B. U., & Ramasami, T. (2008). Aqueous two-phase poly (ethylene glycol)–poly (acrylic acid) system for protein partitioning: Influence of molecular weight, pH and temperature. *Process Biochemistry*, 43(9): 905-911.

Sarubbo, L. A., Oliveira, L. A. D., Porto, A. L. F., Campos-Takaki, G. M. D., & Tambourgi, E. B. (2004). Partition of proteins in aqueous two-phase systems based on cashew-nut tree gum and poly (ethylene glycol). *Brazilian Archives of Biology and Technology*, 47(5): 685-691.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(1): 1-10.

Sattayasai, N. (2012). Protein purification, *Chemical Biology. Prof. Deniz Ekinci (Ed.)*, Khon Kaen University, Thailand, 3-18.

Schlemmer, A. F., Ware, C. F., & Keen, N. T. (1987). Purification and characterization of a pectin lyase produced by *Pseudomonas fluorescens* W51. *Journal of Bacteriology*, 169(10): 4493-4498.

Schnitzhofer, W., Weber, H. J., Vršanská, M., Biely, P., Cavaco-Paulo, A., & Guebitz, G. M. (2007). Purification and mechanistic characterisation of two polygalacturonases from *Sclerotium rolfsii*. *Enzyme and Microbial Technology*, 40(7): 1739-1747.

Scopes, R. K. (2002). *Enzyme Activity and Assays*. Encyclopedia of Life Sciences, New York: Macmillan Publishers Ltd, Nature Publishing Group.

Seddon, A. M., Curnow, P., & Booth, P. J. (2004). Membrane proteins, lipids and detergents: not just a soap opera. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1666(1): 105-117.

Shams, K. A., Abdel-Azim, N. S., Saleh, I. A., Hegazy, M. E. F., El-Missiry, M. M., & Hammouda, F. M. (2015). Green technology: Economically and environmentally innovative methods for extraction of medicinal & aromatic plants (MAP) in Egypt. *Journal of Chemical and Pharmaceutical Research*, 7(5): 1050-1074.

Shanmugam, S. and Sathishkumar, T. (2009) Enzyme technology. New Delhi: I.K International Publishing House PVT.

Shene, C., Monsalve, M. T., Vergara, D., Lienqueo, M. E., & Rubilar, M. (2016). High pressure homogenization of *Nannochloropsis oculata* for the extraction of intracellular components: Effect of process conditions and culture age. *European Journal of Lipid Science and Technology*, 118(4): 631-639.

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.

Shubakov, A. A., & Elkina, E. A. (2002). Production of polygalacturonases by filamentous fungi *Aspergillus niger* ACMF-1119 and *Penicillium dierckxii* ACIMF-152. *Chemistry and Computational Simulation*, 2(7): 65-68.

Shukla, A. A., Etzel, M. R., & Gadam, S. (Eds.). (2006). *Process scale bioseparations for the biopharmaceutical industry*. Boca Raton: CRC Press.

Shukla, A., Gupta, R. C., & Sharma, P. (2013). Comparative study of microwave assisted with conventional extraction of calcium sennosides from senna leaf. *Reasearch Journal of Pharmaceutical Biological and Chemical Science*, 4(3): 103-109.

Siddiqui, M. A., Pande, V., & Arif, M. (2012). Production, purification, and characterization of polygalacturonase from *Rhizomucor pusillus* isolated from decomposting orange peels. *Enzyme Research*, 2012: 1-8.

Sidhu, J. S. (2006). *Tropical fruits: Guava, lychee, and papaya*. Handbook of fruits and fruit processing, 597.

Silvério, S. C., Rodríguez, O., Tavares, A. P., Teixeira, J. A., & Macedo, E. A. (2013). Laccase recovery with aqueous two-phase systems: enzyme partitioning and stability. *Journal of Molecular Catalysis B: Enzymatic*, 87: 37-43.

Silvério, S. C., Wegrzyn, A., Lladosa, E., Rodríguez, O., & Macedo, E. A. (2012). Effect of aqueous two-phase system constituents in different poly (ethylene glycol)–salt phase diagrams. *Journal of Chemical and Engineering Data*, 57(4): 1203-1208.

Singh, D., Singh, R., & Bhatt, F. (2014). Development, quality evaluation and shelf life studies of whey guava beverage. *International Journal of Current Engineering and Technology*, 4(3): 2171-2175.

Singh, J., & Sharma, A. (2012). Application of response surface methodology to the modelling of cellulase purification by solvent extraction.*Advances in Bioscience and Biotechnology*, 3(04): 408-416.

Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian Journal of Pharmacology*, 43(3): 246.

Smith, A. M. (2015). *Interaction of Metal Ions with Proteins as a Source of Inspiration for Biomimetic Materials*. UK: Royal Society of Chemistry.

Soares, M. M., Silva, R. D., & Gomes, E. (1999). Screening of bacterial strains for pectinolytic activity: characterization of the polygalacturonase produced by *Bacillus sp*. *Revista de Microbiologia*, 30(4): 299-303.

Solís, S., Loeza, J., Segura, G., Tello, J., Reyes, N., Lappe, P., Guitérrez, L., Ríos, F., & Huitrón, C. (2009). Hydrolysis of orange peel by a pectin lyase-overproducing hybrid obtained by protoplast fusion between mutant pectinolytic *Aspergillus flavipes* and *Aspergillus niveus* CH-Y-1043. *Enzyme and Microbial Technology*, 44(3): 123-128.

Souza, R. L., Lima, R. A., Coutinho, J. A., Soares, C. M., & Lima, Á. S. (2015). Aqueous two-phase systems based on cholinium salts and tetrahydrofuran and their use for lipase purification. *Separation and Purification Technology*, 155: 118-126.

Steinitz, B. (1999). Sugar alcohols display non-osmotic roles in regulating morphogenesis and metabolism in plants that do not produce polyols as primary photosynthetic products. *Journal of Plant Physiology*, 155(1): 1-8.

Steinitz, B. (1999). Sugar alcohols display nonosmotic roles in regulating morphogenesis and metabolism in plants that do not produce polyols as primary photosynthetic products. *Journal of Plant Physiology*, 155(1): 1-8.

Stone, B. C., (1970). The flora of Guam. *Micronesica* 6: 454–455.

Sun, H., Ge, X., Lv, Y., & Wang, A. (2012). Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in food and feed. *Journal of Chromatography A*, 1237: 1-23.

Suresh, B., & Viruthagiri, T. (2010). Optimization and kinectics of pectinase enzyme using *Aspergillus niger* by solid-state fermentation. *Indian Journal of Science and Technology*, 3(8): 867-870.

Szabo, O. E., Csiszar, E., Toth, K., Szakacs, G., & Koczka, B. (2015). Ultrasound-assisted extraction and characterization of hydrolytic and oxidative enzymes produced by solid state fermentation. *Ultrasonics Sonochemistry*, 22: 249-256.

Tan, P. W., Tan, C. P., & Ho, C. W. (2011). Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga *(Centella asiatica). International Food Research Journal*, 18(2): 557-562.

Tan, S. C., & Yiap, B. C. (2009). DNA, RNA, and protein extraction: the past and the present. *Journal of Biomedicine and Biotechnology*, 2009: 574398-574398.

Tang, M. S., Show, P. L., Lin, Y. K., Woon, K. L., Tan, C. P., & Ling, T. C. (2014). Separation of single-walled carbon nanotubes using aqueous two-phase system. *Separation and Purification Technology*, 125: 136-141.

Taylor, A. J., & Leach, R. M. (1995). Enzymes in the food industry. In: *Enzymes in Food Processing*, Tucker, G.A. and Woods, L.F.J, (Eds.), US: Springer, 26-40.

Teng, H. N., Wang, F., Sun, M. J., & Zhang, S. (2005). Influence of salts on aqueous two-phase system of cationic and anionic surfactant aqueous mixture. *Acta Chimica Sinica Chinese Edition*, 63(17): 1570-1574.

Themmen, A. P., Tucker, G. A., & Grierson, D. (1982). Degradation of isolated tomato cell walls by purified polygalacturonase in vitro. *Plant Physiology*, 69(1): 122-124.

Torimiro, N., & Okonji, R. E. (2013). A comparative study of pectinolytic enzyme production by *Bacillus* species. *African Journal of Biotechnology*, 12(46): 6498-6503.

Torimiro, N., & Okonji, R. E. (2013). A comparative study of pectinolytic enzyme production by *Bacillus* species. *African Journal of Biotechnology*, 12(46): 6498-6503.

Trindade, L. V., Desagiacomo, C., Polizeli, M. D. L. T. D. M., Damasio, A. R. D. L., Lima, A. M. F., Gomes, E., & Bonilla-Rodriguez, G. O. (2016). Biochemical characterization, thermal stability, and partial sequence of a novel exo-polygalacturonase from the thermophilic fungus*Rhizomucor pusillus* A13. 36

obtained by submerged cultivation. *BioMed Research International*, 2016: 1-11.

Ünal, M. Ü., & Şener, A. (2015). Extraction and characterization of pectin methylesterase from Alyanak apricot *(Prunus armeniaca L). Journal of Food Science and Technology*, 52(2): 1194-1199.

Vardanega, R., Santos, D. T., & Meireles, M. A. (2014). Intensification of bioactive compounds extraction from medicinal plants using ultrasonic irradiation. *Pharmacognosy Reviews*, 8(16): 88-95.

Verlent, I., Van Loey, A., Smout, C., Duvetter, T., & Hendrickx, M. E. (2004). Purified tomato polygalacturonase activity during thermal and high-pressure treatment. *Biotechnology and Bioengineering*, 86(1): 63-71.

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.

Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics sonochemistry*, 8(3): 303-313.

Wagner, F., Kusserow, H., & Schäfer, W. (2000). Cloning and targeted disruption of two polygalacturonase genes in *Penicillium olsonii*. *FEMS Microbiology Letters*, 186(2): 293-299.

Wallner, S. J., & Bloom, H. L. (1977). Characteristics of tomato cell wall degradation in vitro implications for the study of fruit-softening enzymes. *Plant Physiology*, 60(2): 207-210.

Wang, K., Yin, H., Sha, W., Huang, J., & Fu, H. (2007). Temperature-sensitive aqueous surfactant two-phase system formation in cationic-anionic surfactant systems. *TheJournal of Physical Chemistry B*, 111(45): 12997-13005.

Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, 17(6): 300-312.

Wang, W. W., Yuan, X. Z., Zeng, G. M., Liang, Y. S., & Chao, Y. (2010). Enzymatic hydrolysis of cellulose in reverse micelles.*Journal of Environmental Science*, 31(9): 2202–2207.

Ward, W. (2012). *The art of protein purification*. Protein Purification, Dr. Rizwan Ahmad (Ed.), USA: INTECH Open Access Publisher.

Weschayanwiwat, P., Kunanupap, O., & Scamehorn, J. F. (2008). Benzene removal from waste water using aqueous surfactant two-phase extraction with cationic and anionic surfactant mixtures. *Chemosphere*, 72(7): 1043-1048.

Wesslén, T., Albertsson, P. Å., & Philipson, L. (1959). Concentration of animal viruses using two-phase systems of aqueous polymer solutions. *Archiv für die gesamte Virusforschung*, 9(4): 510-520.

Wilson, K. J., Nessler, C. L., & Mahlberg, P. G. (1976). Pectinase in *Asclepias latex* and its possible role in laticifer growth and development. *American Journal of Botany*, 1140-1144.

Wu, Y. T., Pereira, M., Venâncio, A., & Teixeira, J. (2000). Recovery of endo-polygalacturonase using polyethylene glycol-salt aqueous two-phase extraction with polymer recycling. *Bioseparation*, 9(4): 247-254.

Wu, Y. T., Pereira, M., Venâncio, A., & Teixeira, J. (2001). Separation of endo-polygalacturonase using aqueous two-phase partitioning. *Journal of Chromatography A*, 929(1): 23-29.

Xia, E. Q., Ai, X. X., Zang, S. Y., Guan, T. T., Xu, X. R., & Li, H. B. (2011). Ultrasound-assisted extraction of phillyrin from *Forsythia suspensa*.*Ultrasonics Sonochemistry*, 18(2): 549-552.

Yadav, P. K., Singh, V. K., Yadav, S., Yadav, K. D. S., & Yadav, D. (2009). In silico analysis of pectin lyase and pectinase sequences. *Biochemistry (Moscow)*, 74(9): 1049-1055.

Yadav, S., & Shastri, N. V. (2005). Partial purification and characterization of a pectin lyase produced by *Penicillium oxalicum* in solid-state fermentation (SSF). *Indian Journal of Biotechnology*, 4(4): 501-505.

Yau, Y. K., Ooi, C. W., Ng, E. P., Lan, J. C. W., Ling, T. C., & Show, P. L. (2015). Current applications of different type of aqueous two-phase systems.*Bioresources and Bioprocessing*, 1(2): 1-13.

Yolmeh, M., Najafi, M. B. H., & Farhoosh, R. (2014). Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). *Food Chemistry*, 155: 319-324.

Yuan, P., Meng, K., Luo, H., Shi, P., Huang, H., Bai, Y., Yang, P., & Yao, B. (2011). A novel low-temperature active alkaline pectate lyase from *Klebsiella sp*. Y1 with potential in textile industry. *Process Biochemistry*, 46(10): 1921-1926.

Yuan, P., Meng, K., Wang, Y., Luo, H., Shi, P., Huang, H., Tu, T., Yang, P., & Yao, B. (2012). A low-temperature-active alkaline pectate lyase from Xanthomonas campestris ACCC 10048 with high activity over a wide pH range. *Applied Biochemistry and Biotechnology*, 168(6): 1489-1500.

Yücekan, İ., & Önal, S. (2011). Partitioning of invertase from tomato in poly (ethylene glycol)/sodium sulfate aqueous two-phase systems. *Process Biochemistry*, *46*(1): 226-232.

Zafarani-Moattar, M. T., & Hamzehzadeh, S. (2007). Liquid-liquid equilibria of aqueous two-phase systems containing 1-butyl-3-methylimidazolium bromide and potassium phosphate or dipotassium hydrogen phosphate at 298.15 K. *Journal of Chemical & Engineering Data*, 52(5): 1686-1692.

Zhang, H. F., Yang, X. H., Zhao, L. D., & Wang, Y. (2009). Ultrasonic-assisted extraction of epimedin C from fresh leaves of *Epimedium* and extraction mechanism. *Innovative Food Science & Emerging Technologies*, 10(1): 54-60.

Zhang, J., Wang, Y., & Peng, Q. (2013). Phase behavior of aqueous two-phase systems of cationic and anionic surfactants and their application to theanine extraction. *Korean Journal of Chemical Engineering*, 30(6): 1284-1288.

Zhang, S. Q., Bi, H. M., & Liu, C. J. (2007). Extraction of bio-active components from Rhodiola sachalinensis under ultrahigh hydrostatic pressure. *Separation and Purification Technology*, 57(2): 277-282.

Zhang, S., Gao, Y., Dong, B., & Zheng, L. (2010). Interaction between the added long-chain ionic liquid 1-dodecyl-3-methylimidazolium tetrafluoroborate and Triton X-100 in aqueous solutions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 372(1): 182-189.

Zhang, Z. S., Wang, L. J., Li, D., Jiao, S. S., Chen, X. D., & Mao, Z. H. (2008). Ultrasound-assisted extraction of oil from flaxseed. *Separation and Purification Technology*, 62(1): 192-198.

Zhao, Q., Yuan, S., Zhang, Y., Zhu, H., Dai, C., Yang, F., & Han, F. (2007). Expression, purification and characterization of pectate lyase A from *Aspergillus nidulans* in *Escherichia coli*. *World Journal of Microbiology and Biotechnology*, 23(8): 1057-1064.

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.

Zohdi, N. M. (2014). *Extraction, purification and characterization of amylase from red pitaya (hylocereus polyrhizus [weber] britton & rose) peel.* Msc Thesis. Universiti Putra Malaysia.

Zou, T. B., Wang, M., Gan, R. Y., & Ling, W. H. (2011). Optimization of ultrasound-assisted extraction of anthocyanins from mulberry, using response surface methodology. *International Journal of Molecular Sciences*,*12*(5): 3006-3017.

Zulkifli, M. B. (2010). *Antioxidant properties of mango, guava and papaya peel extracts and their effects on the stability, physicochemical properties and acceptability of frozen beef burger,* PhD Thesis, Universiti Teknologi MARA.