DEVELOPMENT OF MULTIPLE PROBE CRYO-CONCENTRATION SYSTEM FOR PROGRESSIVE FREEZE CONCENTRATION OF LYSOZYME AQUEOUS SOLUTION

NORSHAFIKA BINTI YAHYA

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy

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

JULY 2020

ACKNOWLEDGEMENTS

In the name of Allah S.W.T. the most gracious and most merciful, Lord of the universe, with His permission Alhamdulillah this thesis has been completed. I would like to express my precious thanks to my supervisor, Associate Professor Dr. Mazura Jusoh and my co-supervisor Ir. Dr. Zaki Yamani Zakaria for their supervision, valuable advice, guidance, support, encouragement and value of friendship that has enabled me to complete this thesis on time. I think, without her support and encouragement, this thesis would not have been completed.

I also wish to express my deepest gratitude to all my colleagues, Nor Zanariah Safie and Nur Nazlina Saimon for their help and those who have been involved directly or indirectly in giving their unselfish advice and assistance in my journey to make this research a great success.

I owe tremendous appreciation to my truly loved husband, Nazrizal Bin Zainal Abidin and my whole family, especially to my beloved parents, Yahya Bin Ya'acob and Fatimah Binti Sitam, my mother and father-in-law Pauziah Wagimin and Zainal Abidin Mohammad whose great generous understanding has supported me in every way. Not forgetting my sisters, and my brothers for their generous support and motivation during the tough times throughout the preparation of this thesis. Thank you so much and may Allah S.W.T. the Almighty be with us all the time.

ABSTRACT

A new crystalliser to concentrate lysozyme aqueous solution through freeze concentration was designed in this study to overcome the shortcomings of the currently available methods in concentrating protein. A new compatible, simple, reliable and low maintenance design was developed in this study based on progressive freeze concentration principles called multiple probe cryo-concentration system (MPCC). Progressive freeze concentration process is a process which rejects all impurities or solute by generating ice crystal lattice from the mother liquor, thus the remaining solution is more concentrated. The aim of this research is to observe the possibility of the new design system in producing high concentration lysozyme aqueous solution according to the four-effect parametric condition which includes coolant temperature, stirrer speed, operation time and initial concentration. The complete design of MPCC system consists of a solution tank with outer tubular cooling jacket and insulator, containing the protein solution and the cooled multiple probes immersed in it. The concentration process began when the temperature of the lysozyme aqueous solution dropped until the ice crystal formed on the wall of the probe while assisted by the stirrer. The concentrated protein solution was then separated from the ice crystal layer formed and collected as product. In order to evaluate the capability of the design, effective partition constant (K), solute yield of lysozyme (Y), concentration index (CI) and average ice growth rate ($\tilde{v}_{ice}$) were analysed using UV-Vis spectrophotometer to determine the solute concentration. The findings revealed that coolant temperature at -12 °C, stirrer speed at 350 rpm, operation time at 40 minutes and initial concentration at 10 mg/ml gave the best result of K, Y and Cl and $\tilde{v}_{ice}$. Meanwhile, the determination of optimum condition by response surface methodology indicated that coolant temperature is the most significant parameter followed by stirrer speed and operation time but initial concentration was found to be not significant in affecting the process for both responses of K-value and Y. A thermodynamic prediction model was also built and its validity for ice crystal growth rate prediction was found to be adequately accurate compared to the actual experimental result based on the error analysis obtaining R-squared of 0.98 and absolute average relative deviation of 8.08 %. The heat transfer analysis discovered that the overall heat transfer coefficient, U_o and heat remover, Q are quite similar for stirrer speed and operation time where increased stirrer speed and lower operation time resulted in lower U_o and Q while increased initial concentration would increase the U_o and Q. Despite, U_o and Q are slightly different and contradict with each other when coolant temperature was increased where U_o would increase but Q was decreased. The results from the analysis and investigation shed light on the theory behind the concentration method of protein using freezing method with the newly designed cryo-concentration device, which has never been investigated, tested and discussed specifically for protein concentration.

ABSTRAK

Satu pengkristal baharu untuk memekatkan larutan berair lysozyme melalui pemekatan beku direka dalam kajian ini untuk mengatasi kekurangan kaedah yang ada sekarang dalam pemekatan protein. Reka bentuk yang serasi, mudah, boleh dipercayai dan berpenyelenggaraan rendah yang baharu telah dibangunkan dalam kajian ini berdasarkan prinsip-prinsip kepekatan pembekuan progresif yang dipanggil pemekat krio berbilang kuar (MPCC). Proses pemekatan beku progresif adalah proses yang menolak semua bendasing atau bahan larut dengan menghasilkan kekisi kristal ais daripada larutan utama, akhirnya meninggalkan larutan yang lebih pekat. Tujuan penyelidikan ini adalah untuk melihat kemungkinan sistem reka bentuk baharu dalam menghasilkan larutan berair lysozyme yang berkepekatan tinggi mengikut empat parameter termasuk suhu penyejuk, kelajuan pengaduk, masa operasi dan kepekatan awal. Reka bentuk lengkap sistem MPCC ini terdiri daripada tangki larutan dengan jaket menyejuk tiub luar dan penebat yang mengandungi larutan protein dan kuar yang disejukkan terendam di dalamnya. Proses pemekatan bermula apabila suhu larutan berair lysozyme turun sehingga kristal ais terbentuk pada dinding kuar sambil dibantu oleh pengaduk. Larutan protein pekat kemudian dipisahkan dari lapisan kristal ais yang terbentuk dan dikumpulkan sebagai produk. Untuk menilai keupayaan reka bentuk, pemalar pemisahan berkesan (K), hasil larutan lysozyme (Y), indeks kepekatan (CI) dan purata kadar pertumbuhan ais ($\tilde{v}_{ice}$) dianalisis dengan menggunakan spektrofotometer UV-Vis untuk menentukan kepekatan larutan. Penemuan menunjukkan bahawa suhu penyejuk pada -12 °C, kelajuan pengadukan pada 350 rpm, masa operasi pada 40 minit dan kepekatan awal pada 10 mg/ml memberikan hasil terbaik K, Y, Cl dan $\tilde{v}_{ice}$. Sementara itu, penentuan keadaan optimum oleh kaedah tindakbalas permukaan menunjukkan bahawa suhu penyejuk adalah parameter yang paling penting diikuti oleh kelajuan pengaduk dan masa operasi tetapi kepekatan awal didapati tidak penting dalam mempengaruhi proses bagi kedua-dua K dan Y. Model ramalan termodinamik juga dibina dan kesahannya untuk ramalan kadar pertumbuhan ais kristal didapati cukup tepat berbanding dengan keputusan eksperimen sebenar berdasarkan analisis ralat yang memperoleh R-kuadrat 0.98 dan sisihan relatif purata mutlak 8.08 %. Analisis pemindahan haba mendapati bahawa pekali pemindahan haba keseluruhan, U_o, dan kehilangan haba, Q, adalah hampir sama untuk kelajuan pengaduk dan masa operasi di mana kelajuan pengaduk yang semakin meningkat dan masa operasi yang lebih rendah memberikan U_o dan Q yang rendah manakala peningkatan kepekatan awal akan meningkatkan U_o dan Q. Walaubagaimanapun, U_o dan Q sedikit berbeza dan bercanggah antara satu sama lain apabila suhu penyejuk dinaikkan di mana U_o akan meningkat tetapi Q berkurangan. Keputusan dari analisis dan penyiasatan menggambarkan teori di sebalik kaedah pemekatan protein menggunakan kaedah pembekuan dengan alat pemekatan krio yang baharu yang direka, yang tidak pernah diselidiki, diuji dan dibincangkan khusus untuk kepekatan protein..

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PFC	-	Progressive freeze concentration
SFC	-	Suspension freeze concentration
MPCC	-	Multiple probe cryo-concentrator
RSM	-	Response surface methodology
UV-vis	-	Ultraviolet-visible spectrophotometry
FC	-	Freeze concentration
BSA	-	Bovine serum albumin
rpm	-	Rotation per minute
CCD	-	Central composite design
ANOVA	-	Analysis of variance
DOE	-	Design of experimental
CTemp	-	Coolant temperature
OpTime	-	Operation time
StSp	-	Stirrer speed
InCon	-	Initial concentration
SST	-	Total sum of square
SSres	-	Residual/error
MS	-	Mass square
Cl	-	Concentration Index

LIST OF SYMBOLS

K	-	Effective partition constant
$\dot{Q}_{LC}$	-	Energy demand
U_o	-	Overall heat transfer coefficient
β	-	Beta
Y	-	Yield
C_L	-	Concentration of liquid
C_s	-	Concentration of solid
V_L	-	Volume of liquid
T	-	Time
ρ_{ice}	-	Density of ice
ρ_{liq}	-	Density of liquid
A	-	Area (cm)
%	-	Percentage
U	-	Rate of crystal front
µ	-	Rate of advance of crystal front
$\dot{m}$	-	Mass flow rate
C_{Fs}	-	Final solute concentration
Cs	-	Concentration of solute
C_{H2O}	-	Concentration of water
C_1	-	Initial solute concentration
M	-	Mass
v_1	-	Initial volume
W	-	Watt
α	-	Alfa
˚C	-	Degree celsius
Θ	-	Entropy production per unit
Q	-	Total heat transfer

J_q	-	Flux of heat
J_{H2O}	-	Mass flux of water
J_s	-	Mass flux of solute
V_{ice}	-	Molar volume of ice
V_{H2O}	-	Molar volume of water
A_m	-	Logarithmic mean area
A_i	-	Inner surface area
A_o	-	Outer surface area
C_i	-	Concentration interface between ice and solution
k_i	-	Kinetic coefficient
K_c	-	Mass transfer coefficient
R	-	Resistance
ω_s	-	Solute mass fraction
δ	-	Film thickness
$\Delta_f H$	-	Enthalpy of freezing
υ_{ice}	-	Velocity of ice growth
$\tilde{\upsilon}_{ice}$	-	Average ice growth rate
q	-	Factor
K	-	Run

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Chemical engineering separation technologies have always demanded for process efficiency, predictability, economic and simplicity aspect, same goes to technology of protein concentration and purification methods. The key is to develop technologies that are transforming and improving the way of innovation due to shortcomings of the existing protein separation methods or techniques. The separation methods of these types of protein for example egg white have been developed since early 1900 but preparation methods of these protein for commercial application are still under research and development. Those sequential preparation techniques of separation for these multiple protein are very important criteria involving simplicity, scalability and use of nontoxic chemicals for further commercial production and application (Abeyrathne *et al.,* 2013a). Freeze concentration is believed to be an applicable method to separate protein due to its simplicity which does not involve toxic chemical despite of its shortcoming, low productivity and low increment of protein separation compared to other methods used in previous research.

In protein purification, one of the steps is to remove water from the protein aqueous solution. It is familiar that protein in aqueous solution is surrounded by water which has different properties from bulk water. The water called hydration water is more difficult to remove from the protein due to low activity (Rickard *et al.*, 2010). Water hydration also has fewer degrees of freedom and a long residence time (Rickard *et al.*, 2010). Other than that, protein destabilization /stabilization also depends on the initial hydration level of protein and the water content in acetonitrile (Sirotkin and Kuchierskaya, 2017).

In order to complete the study about the protein separation of water from protein aqueous solution, hen egg-white lysozyme was used as a model protein in this research due to its high availability in the market, cheapest compared to the other proteins and most importantly, it is applied and studied in biophysical and biotechnological investigation. Lysozyme is well known as an effective immunological agent and an antimicrobial peptide with a high enzymatic activity (Ercan and Demirci, 2016). Large amounts of lysozyme can be found in egg white and several in secretion including tears, saliva, human milk and mucus. Lysozyme is also one of the protein enzymes which have a lot of benefits including bladder health support, healthy inflammation management, infection management, also support for wound repair and has many applications in both food and dietary supplements. Water content surrounding lysozyme can be interpreted in Figure 1. 1. The connected red and white coloured atoms represent molecules of water content, light blue colour represents other substance, meanwhile striped blue colour represents the structure of lysozyme.

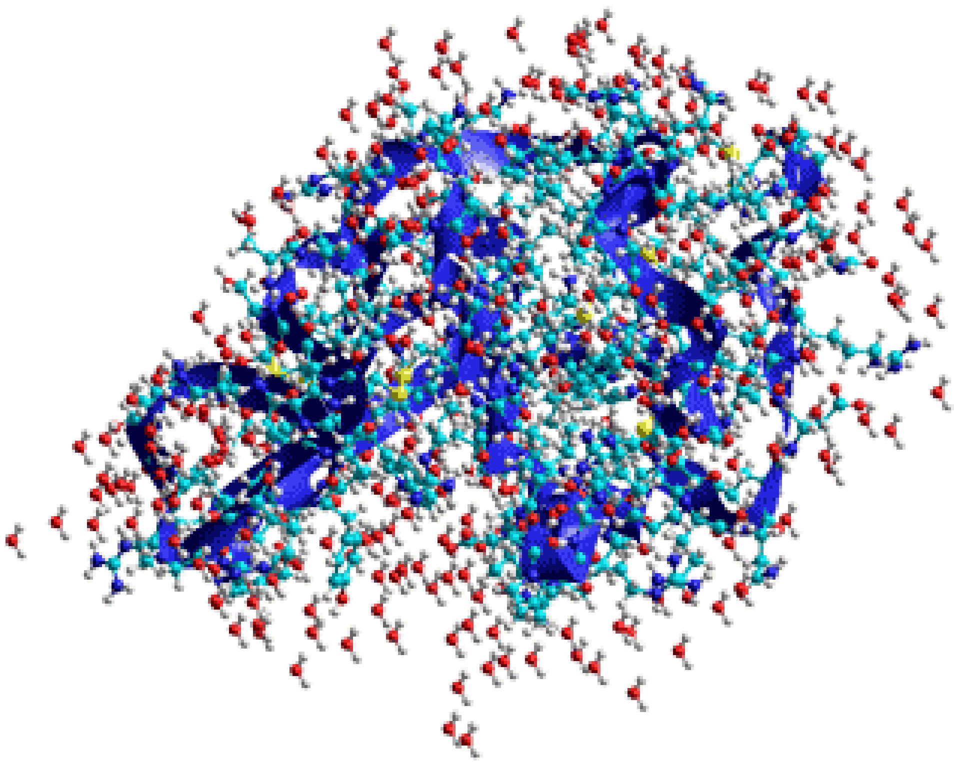

Figure 1.1 Schematic of human lysozyme with water content (Chaplin, 2019).

The process of removing water and concentrating lysozyme aqueous solution is very important in order to make sure the desired concentration is achieved for further application. Major challenges to concentrate the protein are to achieve high end product concentration, better quality with preserved or retained original biological structure of protein and to reduce operation time intended for subcutaneous administration. Most of the methods can achieve approximately in the range of 30 to 40 mg/ml of lysozyme concentration but they are lacking in the maintenance methods (ultrafiltration and dialysis) and they utilize longer time which is more than an hour. Thus, the biochemical companies and especially the researchers are always looking for the best technique or method to achieve a good result of concentration process for further applicable analysis, reaction and administration procedure.

Freeze concentration is a process of concentrating a solution by freezing out the water content into ice crystals. By comparing to evaporation and membrane technology, freeze concentration has given some benefits compared to the others in producing a concentrate with high quality product because the process occurs at low temperature where no vapour or liquid interface exist resulting minimal loss of volatiles (Jusoh *et al.*, 2013). Recently, a new freeze concentration system has been introduced in the configuration of a one-step system, which is progressive freeze concentration (PFC) that is simpler than the conventional suspension freeze concentration (SFC).

Progressive freeze concentration method was applied in this research because the concept of this method can reduce the water content at almost 90% (Miyawaki *et al.*, 2012) and avoid protein denaturation contributed by vigorous heat. Progressive freeze concentration forms a single block of ice compared to the suspension freeze concentration, where small ice crystals are formed suspended in the mother liquor. Hence the final separation of ice and the concentrate becomes easier. Besides, according to Yahya et al. (2017), the advantage of this feature with the large surface area is that the operation becomes easier because the ice lining process can be neglected. Other than that, supercooling can also be avoided, thus preventing operation at super low temperature to accommodate ice nucleation. This will improve its productivity and reduce operation cost (Yahya *et al.*, 2017a).

1.2 Problem Statement

Protein or lysozyme concentration and purification technology is demanding for simplicity, predictability, efficiency, economic and higher increment of concentration process equipment to overcome the shortcomings of the current and previous methods. Although many methods have been used in this particular process, they still do not achieve all the criteria demanded by the industries and community. Thus, this project is really important to study the improvement that will occur during the process using a newly built innovation of technologies.

There are several methods that are commonly available in carrying out protein concentration process namely, lyophilisation, dialysis, cellulose membrane concentrators, precipitation or salting out, chromatography, ultrafiltration and freeze concentration or cryo-concentration, the method has not yet been investigated. The most common method or technique to concentrate protein in small scale that is usually applied is dialysis technique, meanwhile biotech companies which use large scale frequently rely on membrane filtration and evaporation. Literally, the technique of concentrating protein nowadays has many problems and disadvantages including the high cost required in obtaining the osmotic pressure needed and also for membrane replacement attributable from clogging occurrence, the needs for high energy, the fact that the biological structure could also be destroyed due to high temperature applied, high ammonium sulphate concentration may disrupt the biological activity of the protein and may not return on resolubilization, the proteins may interact with the membrane, less productivity and increment of protein concentration. All the problems and disadvantages may cause reduction of protein quality and sometimes highly concentrated protein could not be adequately produced.

In order to solve the mentioned problems, other techniques or methods are needed to provide better alternative and the best choice is using freeze concentration or cryo-concentration method based on other previous researches which provide good results in reducing the number of unit operations with less time consumption. This is also supported by Miyawaki et al. (2016) where cryo-concentration method is the best method among other methods in concentrating liquid food and biochemical solution due to several advantages: ease of separation of concentrated solution due to simple equipment, low energy requirement, relative component-distribution is kept unchanged, the process temperature is low thus preventing undesirable biochemical and chemical changes, also minimal loss of flavour and aromas (Cisse *et al.*, 2011; Gunathilake *et al.*, 2014a; Gunathilake *et al.*, 2014b; Miyawaki *et al.*, 2016a; Miyawaki *et al.*, 2012). Cryo-concentration is a technique by which pure water can be removed from the feed solution and formed into ice, which leaves behind solution with higher concentration.

Although a recent work by Yu et al., 2014 had used the same material and methods called Solvent Freeze-out Technology (SFOT) from the concept of cryo-concentration technique, they had not achieved high quality separation with low productivity which resulted in yield of only 51.8% and collected 0.29 g of lysozyme using more than an hour to complete the process (Yu *et al.*, 2014). Meanwhile, if compared with other designs like Miyawaki et al. (2016), Samsuri et al. (2015) and Amran et al. (2018), those designs are not suitable and have several disadvantages when it deals with the lysozyme aqueous solution concentration process. This is because, the sequence of lysozyme concentration process will firstly go through the process of isolation in which water salt will be mixed with the impure lysozyme as an attempt to purify the lysozyme by precipitating the lysozyme. Hence, a more suitable design is needed as an agitated unit in place of ice freezing to resist from the gravity in order to avoid the precipitated lysozyme from being entrapped in the ice.

Nevertheless, the progressive cryo-concentration still has many advantages and is proposed to be a method to replace and upgrade the previous methods in removing water and concentrating protein solution. Progressive cryo-concentration is a process where ice crystals are formed as a single block of ice on the surface where cooling is supplied to overcome the drawbacks of the existing process to concentrate the protein. The application of the process could make possible concentration of protein because theoretically, the formation of ice crystal lattice rejects all impurities and should then result in high purity of ice, leaving behind highly concentrated protein solution. Another point to be highlighted is the melting point and freezing point of protein is 25°C (Gorania *et al.*, 2010) and -7°C (Wang *et al.*, 2013), respectively. In addition, the progressive cryo-concentration is not affected by the molecular size. This process has never been widely investigated or used in protein concentration before. Thus, it is important to study the concentration effect toward protein solution in order to offer a new technique of producing desirable concentration of antibody protein.

A new design of ice crystalliser for a PFC process called multiple probe cryo-concentrator (MPCC) has been designed and improved with several additional adjustments in this research to figure out the efficiency of the freezing system towards water removal from the lysozyme solution. The new prototype of separation design (MPCC) is believed can increase the concentration of lysozyme and resolved the problems involved in protein separation.

1.3 Objective of the Study

The aim of this research is to provide a new alternative method of lysozyme protein concentration. The objectives of this research include: -

1) To design and fabricate a new crystallizer which is called a multiple probe cryo-concentrator (MPCC) to be applied for lysozyme concentration.

2) To investigate the effect of process condition including coolant temperature, operation time, stirrer speed and initial concentration towards the effective partition constant (K), solute yield (Y), concentration index (Cl) and average ice growth ($\tilde{v}_{ice}$) of lysozyme aqueous solution.

3) To determine the optimum operating parameters in concentration for lysozyme aqueous solution for two responses (K and Y) via MPCC system using Response Surface Methodology (RSM).

4) To analyse the heat and mass transfer for ice formation and perform a thermodynamic modelling of the solution concentration process, considering thermal conductivity of the concentration system.

1.4 Scope of Research

The design of the new crystalliser (MPCC) was considered the element of surface area for heat transfer and freezing process through the introduction of the probe structure, as well as the design of cooling jacket and stirrer for adequate cooling. Meanwhile the geometrical feature of the MPCC was evaluated based on friction profile and comparison on ice productivity and protein concentration.

Probe temperature, operation time, agitation speed and lysozyme aqueous solution concentration was conducted in the range -6 to -12 ˚C, 20 to 60 minutes, 200-400 rpm, and 6 to 14 mg/ml of concentration, respectively.

Lysozyme aqueous solution concentration was analysed by UV-vis spectrophotometer and observed at length in region 280 nm.

Thermodynamic modelling was performed by calculating the rate of heat transfer exchanged in the process and simulated using the parameters.

1.5 Theoretical Framework

The operationalization of the research construct considers two (2) major elements that contribute to the effect of the resulting protein concentration as depicted in Figure 1.2.

:

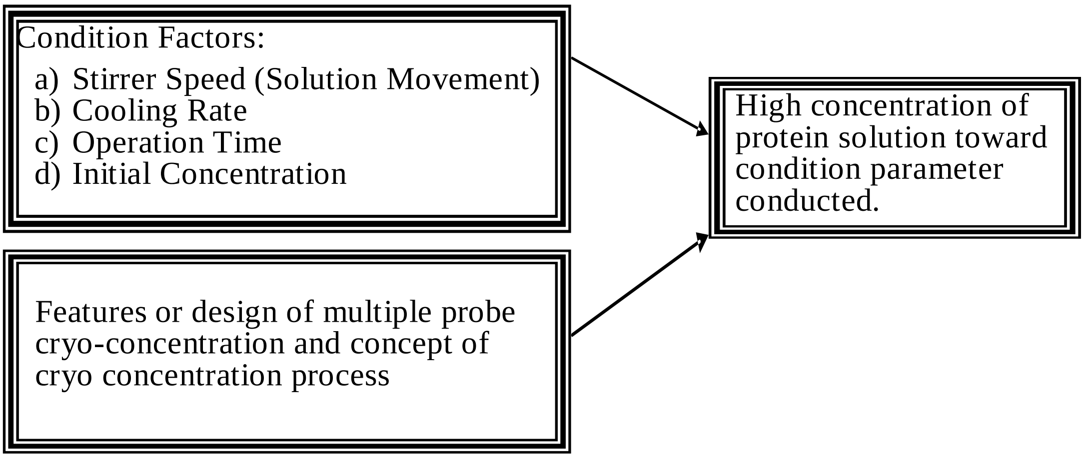

Figure 1.2 Theoretical framework of the study

1.6 Significance of Research

The potential to concentrate a solution at high concentration has always been a priority to apply in small-scale or large-scale. In case of protein solution, the current methods used always create problems that might contribute to low concentration of protein, high cost and maintenance, long process time and affecting the quality of protein concentrate.

However, cryo-concentration can be seen as a way forward or as another alternative method for protein concentration due to the principle of cryo-concentration in which an aqueous solution is concentrated by water freezing and expelling all the impurities. This method has some advantages that contradict the previous existing method. Thus, cryo-concentration method is believed to have potential as a method to concentrate protein solution by using a new crystallizer design from the same concept of cryo-concentration.

.

REFERENCES

Ab Hamid, F. H., Rahim, N. A., Johari, A., Ngadi, N., Zakaria, Z. Y. and Jusoh, M. (2015) 'Desalination of seawater through progressive freeze concentration using a coil crystallizer', *Water Science & Technology: Water Supply*, 15 (3), 625-631. doi:10.2166/ws.2015.019

Abdou, A. M., Kim, M. and Sato, K. (2013) *Functional Proteins Peptides of Hen's Origin*, in Hernandez-Ledesme, B. & Hsieh, C. (eds.) *Bioactive Food Peptides in Health and Disease*. Croatia-European Union: InTech. pp. 115-144.

Abeyrathne, E. D. N. S., Lee, H. Y. and Ahn, D. U. (2013a) 'Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review', *Poultry Science*. 92 (12), 3292-3299.

Abeyrathne, E. D. N. S., Lee, H. Y. and Ahn, D. U. (2014) 'Sequential Separation of Lysozyme, Ovomucin, Ovotransferrin, and Ovalbumin from Egg White', *Poultry Science*, 93 (4), 1001-1009.

Abeyrathne, N. S., Lee, H. Y. and Ahn, D. U. (2013b) 'Sequential separation of lysozyme and ovalbumin from chicken egg white', *Korean Journal for Food Science of Animal Resources*, 33 (4), 501-507.

Aghayari, R., Maddah, H., Ashori, F., Hakiminejad, A. and Aghili, M. (2014) 'Effect of nanoparticles on heat transfer in mini double-pipe heat exchangers in turbulent flow'; *Heat and Mass Transfer*, 51 (3), 301-206.

Akçay, H. and Anagün, A. S. (2013) 'Multi response optimization application on a manufacturing factory', *Mathematical and Computational Applications*, 18 (3), 531-538.

Aminlari, L., Hashemi, M. M. and Aminlari, M. (2014) 'Modified Lysozyme as Novel Broad Spectrum Natural Antimicrobial Agent in Food', *Journal of Food Science*, 79, 1077-1090.

Amran, N. A. and Jusoh, M. (2013) Friction Study on Vertical Finned Crystallizer for Progressive Freeze Concentration System. *Proceedings of the 2013 International Graduate Conference on Engineering Science and Humanity*. Universiti Teknologi Malaysia (UTM), 618-622.

Amran, N. A. and Jusoh, M. (2016) 'Effect of Coolant Temperature and Circulation Flowrate on the Performance of a Vertical Finned Crystallizer', *Procedia Engineering*, 148, 1408-1415.

Amran, N. A., Samsuri, S. and Jusoh, M. (2018) 'Effect of Freezing Time and Shaking Speed on the Performance of Progressive Freeze Concentration via Vertical Finned Crystallizer', *International Journal of Automotive and Mechanical Engineering*, 15 (2), 5356-5366.

Amran, N. A., Samsuri, S., Safiei, N. Z., Zakaria, Z. Y. and Jusoh, M. (2016) 'Review: Parametric Study on the Performance of Progressive Cryoconcentration System', *Chemical Engineering Communications*, 203 (7), 957-975.

Arabski, M., Konieczna, I., Tusi ska, E., W sik, S., Relich, I., Zaj c, K., Kami ski, Z. J. and Kaca, W. (2015) 'The Use of Lysozyme Modified with Fluorescein for the Detection of Gram-positive Bacteria', *Microbiology Research*, 170, 242-247.

Armenante, P. M. and Chang, G.-M. (1998) 'Power Consumption in Agitated Vessels Provided with Multiple-Disk Turbines', *Industrial & Engineering Chemistry Research*, 37 (1), 284-291.

Awade, A. C. and Efstahhion, T. (1999) 'Comparison of Three Liquid Chromatography Methods for Egg-White Protein Analysis', *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 723, 69-74.

Aydar, A. Y. (2018) *Utilization of Response Surface Methodology in Optimization of Extraction of Plant Materials*, in Silva, V. (eds.) *Statistical Approaches With Emphasis on Design of Experiments Applied to Chemical Processes*. Europe: InTechOpen, pp. 157-169.

Ballentine, R. and Gregg, J. R. (1947) 'Determination of Nitrogen. Use of Potassium Biodate in the Iodometric Titration of Ammonia', *Analytical Chemistry*, 19 (4), 281-283.

Bayarri, M., Oulahal, N., Degraeve, P. and Gharsallaoui, A. (2014) 'Properties of lysozyme/low methoxyl (LM) pectin complexes for antimicrobial edible food packaging', *Journal of Food Engineering*, 131, 18-25.

Behera, S. K., Meena, H., Chakraborty, S. and Meikap, B. C. (2018) 'Application of response surface methodology (RSM) for optimization of leaching parameters for ash reduction from low-grade coal', *International Journal of Mining Science and Technology*, 28 (4), 621-629.

Benallou, A. (2018) *Energy and Mass Transfer: Balance Sheet Approach and Basic Concepts*. New Jersey, United State: John Wiley & Sons. Inc.

Bermingham, S. K., Neumann, A. M., Kramer, H. J. M., Verheijen, P. J. T., van Rosmalen, G. M. and Grievink, J. (2000) 'A Design Procedure and Predictive Models for Solution Crystallisation Process', *AlChe Symposion Series*, 323 (96), 250-264.

Bezerra, M. A., Oliveira, E. P., Villar, L. S. and Escaleira, L. A. (2008) 'Response surface methodology (RSM) as a tool for optimization', *Talanta*, 76 (5), 965-977.

Box, G. E. P. and Draper, N. R. (1987) *Empirical model-building and response surfaces*. Oxford, England: John Wiley & Sons.

Box, G. E. P. and Wilson, K. B. (1951) 'On the Experimental Attainment of Optimum Conditions', *Journal of Royal Statical Society (Series B)*, 13 (1), 1-45.

Bradley, N. (2007) *The Response Surface Methodology*. Master of Science, Indiana University South Bend, United Stated of America (USA).

Brown, K. (2019). Alexander Fleming. *Britannica*. Retrieved 14 September 2019, from https://www.britannica.com/biography/Alexander-Fleming

Cai, M., Wang, S. and Liang, H.-h. (2012) 'Optimization of ultrasound-assisted ultrafiltration of Radix astragalus extracts with hollow fiber membrane using response surface methodology', *Separation and Purification Technology*, 100, 74-81.

Callawaert, L. and Michiels, C. W. (2010) 'Lysozyme in the Animal Kingdom', *Journal of Bioscience*, 35, 127-160.

Caretta, O., Courtot, F. and Davies, T. (2006) 'Measurement of salt entrapment during the directional solidification of brine under forced mass convection', *Journal of Crystal Growth*, 294 (2), 151-155.

Carley, K. M., Kamneva, N. Y. and J., R. (2004) *Responce Surface Methodology*: Carnegie Mellon University. pp. 1-26.

Carrillo, W., Garcia-Ruiz, A., Recio, I. and Moreno-Arribas, M. V. (2014) 'Antibacterial Activity of Hen Egg White Lysozyme Modified by Heat and Enzymatic Treatments Against Oenological Lactic Acid Bacteria and Acetic Acid Bacteria', *Journal of Food Protection*, 77, 1732-1739.

Carrillo, W., Tubon, J. and Vilcacundo Chamorro, R. (2016) 'Isolation of hen egg white lysozyme by cation exchange chromatography, analysis of its digestibility and evaluation of the inhibition lipid peroxidation in the zebrafish model', *Asian Journal of Pharmaceutical and Clinical Research*, 9 (3), 345-349.

Chamoli, S. (2015) 'ANN and RSM approach for modeling and optimization of designing parameters for a V down perforated baffle roughened rectangular channel', *Alexandria Engineering Journal*, 54 (3), 429-446.

Chaplin, M. (2019). Protein Hydration. *Water Structure and Science*. Retrieved 15 October 2019, from http://www1.lsbu.ac.uk/water/protein_hydration.html

Chen, P. and Chen, X. D. (2000) 'A generalized correlation of solute inclusion in ice formed from aqueous solutions and food liquids on sub-cooled surface', *The Canadian Journal of Chemical Engineering*, 78 (2), 312-319.

Chen, P., Chen, X. D. and Free, K. W. (1996) 'Measurement and data interpretation of the freezing point depression of milks', *Journal of Food Engineering*, 30 (1), 239-253.

Chen, P., Chen, X. D. and Free, K. W. (1998) 'Solute inclusion in ice formed from sucrose solutions on a sub-cooled surface—an experimental study', *Journal of Food Engineering*, 38 (1), 1-13.

Chen, X. D., Chen, P. and Free, K. W. (1997) 'A Note on the Two Models of Ice Growth Velocity in Aqueous Solutions Derived from an Irreversible Thermodynamics Analysis and the Concentional Heat and Mass Transfer Theory', *Journal of Food Engineering*, 31 (3), 395-402.

Chen, Y. H., Cao, E. and Cui, Z. F. (2001) 'An Experimental Study of Freeze Concentration in Biological Media', *Food and Bioproducts Processing*, 79 (1), 35-40.

Cheng, Y.-C., Lobo, R. F., Sandler, S. I. and Lenhoff, A. M. (2006) 'Kinetics and Equilibria of Lysozyme Precipitation and Crystallization in Concentrated Ammonium Sulfate Solutions', *Biotechnology and Bioengineering*, 94 (1), 177-188.

Cho, S.-R. and Lee, S. (2015) 'A prediction method of ice breaking resistance using a multiple regression analysis', *International Journal of Naval Architecture and Ocean Engineering*, 7 (4), 708-719.

Cisse, M., Vaillant, F., Bouquet, S., Pallet, D., Lutin, F., Reynes, M. and Dornier, M. (2011) 'Athermal Concentration by Osmosis Evaporation of Roselle Extract, Apple and Grape Juice and Impact on Quality', *Innovative Food Science and Emerging Technologies*, 12, 352-360.

Coleman, D. E. and Montgomery, D. C. (1993) 'A Systematic Approach to Planning for a Designed Industrial Experiment', *Technometrics*, 35 (1), 1-12.

Cosentino, C., Paolino, R., Musto, M. and Freschi, P. (2015) *Innovative Use of Jenny Milk from Sustainable Rearing*, in Vastola, A. (eds.) *The Sustainability of Agro-Food and Natural Resource Systems in the Mediterranean Basin*. Cham: Springer International Publishing, pp. 113-132.

Couper, J. R., Penney, W. R., Fair, J. R. and Walas, S. M. (2010) *Mixing and Agitation*, in Couper, J. R., Penney, W. R., Fair, J. R. & Walas, S. M. (eds.), *Chemical Process Equipment (Revised Second Edition)*. Boston: Gulf Professional Publishing, pp. 273-324.

Curtis, J. E., McAuley, A., Nanda, H. and Krueger, S. (2012) 'Protein structure and interactions in the solid state studied by small-angle neutron scattering', *Faraday Dicsussion*, 158, 285-299.

Datta, D., Bhattacharjee, S., Nath, A., Das, R., Bhattacharjee, C. and Datta, S. (2009) 'Separation of ovalbumin from chicken egg white using two-stage ultrafiltration technique', *Separation and Purification Technology*, 66 (2), 353-361.

de Lathouder, K. M., Marques Fló, T., Kapteijn, F. and Moulijn, J. A. (2005) 'A novel structured bioreactor: Development of a monolithic stirrer reactor with immobilized lipase', *Catalysis Today*, 105 (3), 443-447.

Desert, C., Guérin-Dubiard, C., Nau, F., Jan, G., Val, F. and Mallard, J. (2001) 'Comparison of Different Electrophoretic Separation of Hen Egg White Proteins', *Journal of Agricultural and Food Chemistry*, 49 (10), 4553-4561.

Dewangan, A. K., Patel, A. D. and Bhadania, A. G. (2015) 'Stainless Steel for Dairy and Food Industry: A Review', *Journal of Material Science & Engineering*, 4 (5), 1-4.

Ding, Z., Qin, F. G. F., Yuan, J., Huang, S., Jiang, R. and Shao, Y. (2019) 'Concentration of apple juice with an intelligent freeze concentrator', *Journal of Food Engineering*, 256, 61-72.

Duong-Ly, K. C. and Gabelli, S. B. (2014) *Chapter Seven - Salting out of Proteins Using Ammonium Sulfate Precipitation*, in Jon, L. (ed.) *Methods in Enzymology*. Academic Press, 541, pp. 85-94.

Ehrlich, L. E., Feig, J. S. G., Schiffres, S. N., Malen, J. A. and Rabin, Y. (2015) 'Large Thermal Conductivity Differences between the Crystalline and Vitrified States of DMSO with Applications to Cryopreservation', *PLoS One*, 10 (5), e0125862-e0125862. doi.org/10.1371/journal.pone.0125862.

Ejikeme, P. C. N., Onukwuli, D. O. and Ejikeme, E. M. (2014) Optimization of Chemical Treatment Condition for Adenia Lobata Fiber using CCD. *Proceedings of the 2014 International Journal of Engineering and Innovative Technology (IJEIT)*, 23-32.

Elkamel, A., Fatoni, R. and Simon, L. (2012) Optimal Product Design of Wheat Straw Polypropylene Composites. *Proceedings of the 2012 Proceeding of the 2012 International Conference on Industrial Engineering and Operations Management.* 3-6 July. Istanbul, Turkey, 2116-2126.

Eng, K. (2013) *Process Characterization Using Response Surface Methodology*. Bachelor of Science, Statistics. California Polytechnic State University, San Luis Obispo.

Eppler, A., Weigandt, M., Schulze, S., Hanefeld, A. and Bunjes, H. (2011) 'Comparison of different protein concentration techniques within preformulation development', *International journal of pharmaceutics*, 421 (1), 120-129.

Ercan, D. and Demirci, A. (2016) 'Recent advances for the production and recovery methods of lysozyme', *Critical Reviews in Biotechnology*, 36 (6), 1078-1088.

Erdemir, D., Lee, A. Y. and Myerson, A. S. (2019) *Crystal Nucleation*, in Lee, A. Y., Myerson, A. S. & Erdemir, D. (eds.) *Handbook of Industrial Crystallization*. (3rd edition). Cambridge: Cambridge University Press , pp. 76-114.

Ezgi, C. n. (2017). *Chapter 2-Basic Design Methods of Heat Exchanger*, in Murshed, S. M. S. & Lopes, M. M. (eds.) *Heat Exchangers - Design, Experiment and Simulation*. United Kingdom: IntechOpen, pp. 9-35.

Farah Hanim, A. H., Norfatiha, A. R., Ngadi, N., Zakaria, Z. Y. and Mazura, J. (2015) 'Effect of Coolant Temperature on Desalination Process via Progressive Freeze Concentration', *Applied Mechanics and Materials*, 695, 443-446.

Fleming, A. and V.D., A. (1922) 'Observation on a Bacteriolytic Substance ("Lysozyme") Found in Secretions and Tissues', *British Journal of Experimental Pathology*, 3 (5), 252-260.

Flesland, O. (1995) 'Freeze Concentratton by Layer Crystallization', *Drying Technology*, 13 (8-9), 1713-1739.

Førland, K. S., Førland, T. and Ratkje, S. K. (1988). *Irreversible thermodynamics: theory and applications*. Wiley.

Gai, Q. Q., Q, F., Liu, Z. J., Dai, R. J. and Zhang, Y. K. (2010) 'Superparamagnetic lysozyme surface-imprinted polymer prepared by atom transferradical polymerization and its application for protein separation', *Journal of Chromatography A*, 1217 (31), 5035-5042.

Garcia-Ruiz, J. M. (2003) 'Counterdiffusion methods for macromolecular crystallization', *Methods in Enzymol*, 368, 130-154.

Ghelich, R., Jahannama, M. R., Abdizadeh, H., Torknik, F. S. and Vaezi, M. R. (2019) 'Central composite design (CCD)-Response surface methodology (RSM) of effective electrospinning parameters on PVP-B-Hf hybrid nanofibrous composites for synthesis of HfB2-based composite nanofibers', *Composites Part B: Engineering*, 166, 527-541.

Gilbert, S. W. (1991)' Melt Crystallization: Process Analysis and Optimization', *AlChe Journal*, 37 (8), 1205-1218.

Gorania, M., Seker, H. and Haris, P. I. (2010) Predicting a protein's melting temperature from its amino acid sequence. *Proceedings of the 2010 Annual International Conference of the IEEE Engineering in Medicine and Biology*. Aug. 31 2010-Sept. 4 2010, 1820-1823.

Gronski, P., Seiler, F. R. and Schwick, H. G. (1991) 'Discovery of Antitoxins and Development of Antibody Preparation for Clinical Uses from 1890 to 1990', *Molwcular Immunology*, 28, 1321-1332.

Group, E. H. E. a. D. (2018). Guidelines on the Materials of Construction for Equipment in Contact with Food. *EHEDG*. Retrieved October 15, 2019, from https://www.ehedg.org/guidelines/

Gulfo, R., Auleda, J. M., Moreno, F. L., Ruiz, Y., Hernández, E. and Raventós, M. (2014a) 'Multi-plate freeze concentration: Recovery of solutes occluded in the ice and determination of thawing time', *Food Science and Technology International*, 20 (6), 405-419.

Gulfo, R., Auleda, J. M., Raventós, M. and Hernández, E. (2014b) 'Calculation process for the recovery of solutes retained in the ice in a multi-plate freeze concentrator: Time and concentration', *Innovative Food Science & Emerging Technologies*, 26, 347-359.

Gunathilake, M., Dozen, M., Shimmura, K. and Miyawaki, O. (2014a) 'An apparatus for partial ice-melting to improve yield in progressive freeze-concentration', *Journal of Food Engineering*, 142, 64-69.

Gunathilake, M., Simmura, K., Dozen, M. and Miyawaki, O. (2014b) 'Flavor Retention in Progressive Freeze-Concentration of Coffee Extract and Pear (La France) Juice Flavor Condensate', *Food Science and Technology Research*, 20, 547-554.

Habib, B. and Farid, M. (2008) 'Freeze concentration of milk and saline solutions in a liquid–solid fluidized bed: Part II. Modelling of ice removal', *Chemical Engineering and Processing: Process Intensification*, 47 (4), 539-547.

Halde, R. (1980) 'Concentration of impurities by progressive freezing', *Water Research*, 14 (6), 575-580.

Hartel, R. W. and Chung, M. S. (1993) 'Contact Nucleation of Ice in Fluid Dairy Products', *Journal of Food Engineering*, 18 (3), 281-296.

Hartel, R. W. and Espinel, L. A. (1993) 'Freeze Concentration of Skim Milk', *Journal of Food Engineering*, 20 (2), 101-120.

Hasan, M. and Louhi-Kultanen, M. (2015) 'Ice growth kinetics modeling of air-cooled layer crystallization from sodium sulfate solutions', *Chemical Engineering Science*, 133, 44-53.

Hasan, M. and Louhi-Kultanen, M. (2016) 'Water purification of aqueous nickel sulfate solutions by air cooled natural freezing', *Chemical Engineering Journal*, 294, 176-184.

Hasan, M., Rotich, N., John, M. and Louhi-Kultanen, M. (2017) 'Salt recovery from wastewater by air-cooled eutectic freeze crystallization', *Chemical Engineering Journal*, 326, 192-200.

Healthcare, G. (2010) *Prinsiple and Method*, in Biosciences, A. (ed.) *Ion Exchange Chromatography and Chromatofocusing*. Sweden: Imagination at Work, pp. 11-184.

Iqbal, A. A. and A., K. A. (2010) 'Modeling and Analysis of MRR, EWR and Surface Roughness in EDM Milling through Response Surface Methodology', *Journal of Engineering and Applied Sciences*, 5 (2), 154-162.

Irani, V., Guy, A. J., Andrew, D., Beeson, J. G., Ramsland, P. A. and Richards, J. S. (2015) 'Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases', *Molecular Immunology*, 67 (2, Part A), 171-182.

Iritani, E., Katagiri, N., Okada, K., Cao, D.-Q. and Kawasaki, K. (2013) 'Improvement of concentration performance in shaking type of freeze concentration', *Separation and Purification Technology*, 120, 445-451.

Isa, A. A., Samsuri, S. and Amran, N. A. (2019) 'Integration of Maceration and Freeze Concentration for Recovery of Vitamin C from Orange Peel Waste', *IOP Conference Series: Earth and Environmental Science*, 268 (1), 1-7.

James, B. (2015) Mixing 101: Optimal Tank Design. *Understaning How Tank Design Affects Mixing*. Retrieved February 2, 2018, 2018, from https://www.dynamixinc.com/optimal-tank-design.

Jentzer, J.-B., Alignan, M., Vaca-Garcia, C., Rigal, L. and Vilarem, G. (2015) 'Responsction Surface Methodology to Optimise Accelerated Solvent Extraction of Steviol Glycosides from Stevia Rebaudiana Bertoni Leaves', *Food Chemistry*, 166 (0), 561-567.

Jie, W. (2001) 'Solute redistribution and segregation in solidification processes', *Science and Technology of Advanced Materials*, 2 (1), 29-35.

Jindal, B. K. (1972) 'Effect of Vibration on the Segragation of NcCI During Freezing NaCI-Aqueous Solutins', *Journal of Crystal Growth*, 16, 280-282.

Joglekar, A. M. and May, A. T. (1987) 'Product Excellence through Design of Experiments', *Cereal Food World*, 32, 857-868.

John, M., Suominen, M., Kurvinen, E., Hasan, M., Sormunen, O.-V., Kujala, P., Mikkola, A. and Louhi-Kultanen, M. (2019) 'Separation efficiency and ice strength properties in simulated natural freezing of aqueous solutions', *Cold Regions Science and Technology*, 158, 18-29.

Jusoh, M. (2010) *Development of a Novel System for Progressive Freeze Concentration Process.* Doctor of Philosophy (Chemical Engineering), Universiti Teknologi Malaysia, UTM, Skudai, Malaysia.

Jusoh, M., Johari, A., Ngadi, N. and Zakaria, Z. Y. (2013) 'Process Optimization of Effective Partition Constant in Progressive Freeze Concentration of Wastewater', *Advances in Chemical Engineering and Science*, 3 (4), 286-293.

Jusoh, M., Mohamed, N. N., Yahya, N., Musa, R., Mohamad, Z., Ngadi, N., Rahman, R. A., Johari, A. and Zakaria, Z. Y. (2018) 'Process optimisation of effective partition constant in coconut water via progressive freeze concentration', *Chemical Engineering Transactions*, 63, 403-408.

Jusoh, M., Mohd Yunus, R. and Abu Hassan, M. A. (2008) 'Effect of Initial Concentration of Solution and Coolant Temperature on A New Progressive Freeze Concentration System', *Journal of Chemical and Natural Resource Engineering*, 122-129.

Jusoh, M., Mohd Yunus, R. and Abu Hassan, M. A. (2009) 'Development of a New Crystallisation Chamber for a Progressive Freeze Concentration System', *Recent Advance in Technologies*, Chapter 33, 587-600.

Jusoh, M., Ngadi, N., Johari, A. and Zakaria, Z. Y. (2014) Prediction of crystal mass through heat transfer study for a progressive freeze concentration system *Proceedings of the 2014 In Chemeca: Processing excellence: Powering our future* Australia, 679-690.

Kanmani, P., Karthik, S., Aravind, J. and Kumaresan, K. (2013) 'The Use of Response Surface Methodology as a Statistical Tool for Media Optimization in Lipase Production from the Dairy Effluent Isolate Fusarium solani', *ISRN Biotechnology*, 2013, 8.

Kashid, M., Renken, A. and Kiwi-Minsker, L. (2011) 'Mixing efficiency and energy consumption for five generic microchannel designs', *Chemical Engineering Journal*, 167 (2), 436-443.

Keshani, S., Luqman Chuah, A., Nourouzi, M. M., Russly, A. R. and Jamilah, B. (2010) 'Optimization of Concentration Process on PomeloFruit Juice Using Response Surface Methodology (RSM)', *International Food Research Journal*, 17, 733-742.

Lee, A. Y., Erdemir, D. and Myerson, A. S. (2019). *Crystals and Crystal Growth*, in Lee, A. Y., Myerson, A. S. & Erdemir, D. (eds.) *Handbook of Industrial Crystallization* (3 edition). Cambridge: Cambridge University Press, pp. 32-75.

Lee, W. C., Yusof, S., Hamid, N. S. A. and Baharin, B. S. (2006) 'Optimizing Condition for Enzymatic Clarification of Banana Juice Using Response Surface Methodology (RSM)', *Journal of Food Engineering*, 73, 55-63.

Leśnierowski, G. and Cegielska-Radziejewska, R. (2012) 'Potential possibilities of production, modification and practical application of lysozyme', *Acta Scientiarum Polonorum. Technologia Alimentaria*, 11 (3), 223-230.

Liu, H. F., Ma, J., Winter, C. and Bayer, R. (2010) 'Recovery and purification process development for monoclonal antibody production', *mAbs*, 2 (5), 480-499.

Liu, L., Fujii, T., Hayakawa, K. and Miyawaki, O. (1998) 'Prevention of Initial Supercooling in Progressive Freeze-concentration', *Bioscience, Biotechnology, and Biochemistry*, 62 (12), 2467-2469.

Liu, L., Miyawaki, O. and Hayakawa, K. (1999) 'Progressive Freeze Co.centration of Tomato Juice', *Food Science and Technology International*, 5, 108-112.

Liu, L., Miyawaki, O. and Nakamura, K. (1997) 'Progressive Freeze-Concentration of Model Liquid Food', *Food Science and Technology International, Tokyo*, 3 (4), 348-352.

Loeschan, D. (2019). What is The Difference Between Axial and Radial Flow Impellers. *Mixer Direct*. Retrieved April 4, 2020, from https://www.mixerdirect.com/blogs/mixer-direct-blog/what-is-the-difference-between-axial-and-radial-flow-impellers

Lovrien, R. and Matulis, D. (1995) 'Assays for Total Protein', *Current Protocols in Protein Science*, 1 (1), 3.4.1-3.4.24.

Lucas, S., Calvo, M. P., Palencia, C. and Cocero, M. J. (2004) 'Mathematical model of supercritical CO_2 adsorption on activated carbon: Effect of operating conditions and adsorption scale-up', *The Journal of Supercritical Fluids*, 32 (1), 193-201.

Luo, C.-s., Chen, W.-w. and Han, W.-f. (2010) 'Experimental study on factors affecting the quality of ice crystal during the freezing concentration for the brackish water', *Desalination*, 260 (1–3), 231-238.

Maddah, H., Alizadeh, M., Ghasemi, N. and Wan Alwi, S. R. (2014) 'Experimental study of Al2O3/water nanofluid turbulent heat transfer enhancement in the horizontal double pipes fitted with modified twisted tapes', *International Journal of Heat and Mass Transfer*, 78, 1042-1054.

Mahanta, S., Paul, S., Srivastav, A., Pastor, A., Kundu, B. and Chaudhuri, T. K. (2015) 'Stable Self-assembled Nanostructured Hen Egg White Lysozyme Exhibits Strong Anti-Proliferative Activity Against Breast Cancer Cells', *Colloids and Surface B*, 130, 237-245.

Mamat, H., Aini, I., Mamot, S. and Yusof, M. (2002) 'The effect of different types of stirrer and fractionation temperatures during fractionation on the yield, characteristics and quality of oleins', *Journal of Food Lipids - J FOOD LIPIDS*, 9, 295-307.

Mansoury, D., Ilami Doshmanziari, F., Kiani, A., Chamkha, A. and Sharifpur, M. (2018) 'Heat transfer and flow characteristics of Al 2 O 3 /water nanofluid in various heat exchangers: Experiments on counter flow', *Heat Transfer Engineering*, 1-36.

Martel, C. J. (2000) 'Influence of Dissolved Solids on the Mechanism of Freeze-Thaw Conditioning', *Water Resources Research*, 34 (2), 657-662.

Matsuda, A., Kawasaki, K. and Kadota, H. (1999) ' Freeze concentration with supersonic radiation under constant freezing rate - Effect of kind and concentration of solutes', *Journal of Chemical Engineering of Japan*, 32 (5), 569.

McCabe, W. L., Smith, J. C. and Harriott, P. (2001). *Unit Operation of Chemical Engineering*. New York: McGraw-Hill.

Mersmann, A. (1995). *Crystallisation Technology Hanbook*. New York, USA: Marcel Dekkers.

Miron, S. M., Dutournié, P., Thabet, K. and Ponche, A. (2019) 'Filtration of protein-based solution with ceramic ultrafiltration membrane. Study of selectivity, adsorption and protein denaturation', *Comptes Rendus Chimie*, 198-205.

Miyawaki, O. (2001) 'Analysis and Control of Ice Crystal Structure in Frozen Food and Their Application to Food Processing', *Food Science and Technology Research*, 7 (1), 1-7.

Miyawaki, O., Gunathilake, M., Omote, C., Koyanagi, T., Sasaki, T., Take, H., Matsuda, A., Ishisaki, K., Miwa, S. and Kitano, S. (2016a) 'Progressive freeze-concentration of apple juice and its application to produce a new type apple wine', *Journal of Food Engineering*, 171, 153-158.

Miyawaki, O., Kato, S. and Watabe, K. (2012) 'Yield improvement in progressive freeze-concentration by partial melting of ice', *Journal of Food Engineering*, 108 (3), 377-382.

Miyawaki, O., Liu, L., Shirai, Y., Sakashita, S. and Kagitani, K. (2005) 'Tubular ice system for scale-up of progressive freeze-concentration', *Journal of Food Engineering*, 69 (1), 107-113.

Miyawaki, O., Omote, C., Gunathilake, M., Ishisaki, K., Miwa, S., Tagami, A. and Kitano, S. (2016b) 'Integrated system of progressive freeze-concentration combined with partial ice-melting for yield improvement', *Journal of Food Engineering*, 184, 38-43.

Moerman, F. and Partington, E. (2013) 'Materials of Construction for Food Equipment and services: Requirements, Strengths and Weakness', *Journal of Hygienic Engineering and Design*, 10-37.

Moffitt Schall, J. and Myerson, A. S. (2019) *Solutions and Solution Properties,* in Lee, A. Y., Myerson, A. S. & Erdemir, D. (eds.) *Handbook of Industrial Crystallization* (3 edition). Cambridge: Cambridge University Press, pp. 1-31.

Monkos, K. (1997) 'Concentration and temperature dependence of viscosity in lysozyme aqueous solutions', *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology*, 1339 (2), 304-310.

Montgomery, D. M., Opeck, E. A. and Vining, G. G. (2001) Introduction to Linear Regression Analysis, in 3rd Edition, J. W. S. (ed.). New York.

More, A. (2019). Lysozyme Market Share, Size 2019 Global Industry Growth Analysis, Segmentation, Trend, Future Demand and Leading Players Updates by Forecast to 2024 Retrieved 3.4.2020, 2020

Moreno, F. L., Hernández, E., Raventós, M., Robles, C. and Ruiz, Y. (2014a) 'A process to concentrate coffee extract by the integration of falling film and block freeze-concentration', *Journal of Food Engineering*, 128, 88-95.

Moreno, F. L., Quintanilla-Carvajal, M. X., Sotelo, L. I., Osorio, C., Raventós, M., Hernández, E. and Ruiz, Y. (2015a) 'Volatile compounds, sensory quality and ice morphology in falling-film and block freeze concentration of coffee extract', *Journal of Food Engineering*, 166, 64-71.

Moreno, F. L., Raventós, M., Hernández, E. and Ruiz, Y. (2014b) 'Behaviour of falling-film freeze concentration of coffee extract', *Journal of Food Engineering*, 141, 20-26.

Moreno, F. L., Raventós, M., Hernández, E., Santamaría, N., Acosta, J., Pirachican, O., Torres, L. and Ruiz, Y. (2015b) 'Rheological Behaviour, Freezing Curve, and Density of Coffee Solutions at Temperatures Close to Freezing', *International Journal of Food Properties*, 18 (2), 426-438.

Moreno, F. L., Robles, C. M., Sarmiento, Z., Ruiz, Y. and Pardo, J. M. (2013) 'Effect of separation and thawing mode on block freeze-concentration of coffee brews', *Food and Bioproducts Processing*, 91 (4), 396-402.

Moss, D. R. (2004) '*Related Equipment*, in Moss, D. R. (ed.) *Pressure Vessel Design Manual. (Third Edition)*. Burlington: Gulf Professional Publishing, pp. 291-364.

Myers, J. L. and Well, A. D. (2003). *Research Design and Statistical Analysis*. United Stated of America: Lawrence Erlbaum Associates.

Myers, R. H., Montgomery, D. C. and Anderson-Cook, C. M. (2009) *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*. Wiley.

Nakagawa, K., Maebashi, S. and Maeda, K. (2010a) 'Freeze-thawing as a path to concentrate aqueous solution', *Separation and Purification Technology*, 73 (3), 403-408.

Nakagawa, K., Nagahama, H., Maebashi, S. and Maeda, K. (2010b) 'Usefulness of solute elution from frozen matrix for freeze-concentration technique', *Chemical Engineering Research and Design*, 88 (5–6), 718-724.

Nehete, J. Y., S., B. R., Narkhede, M. R. and Gawali, S. R. (2013) 'Natural Protein: Sources,Isolation, Characterization and Application', *Pharmacognosy Review*, 7, 107-116.

Nora'aini, A., Hamzah, S., Ali, A. and Wahab, M. (2017) 'Preparation and Characterization of Asymmetric Ultrafiltration Membrane for Lysozyme Separation: Effect of Polymer Concentration', *Journal of Applied Membrane Science & Technology*, 11.

Nyvit, J. (1968) 'Kinetic of Nucleation in Solutions', *Journal of Crystal Growth*, 3-3, 377-383.

Ojeda, A., Moreno, F., Y. Ruiz, R., Abellán, M., Raventós, M. and Hernandez, E. (2017) 'Effect of Process Parameters on Progressive Freeze Concentration of Sucrose Solutions', *Chemical Engineering Communications*, 204 (8), 951-956.

Omana, D. A., Wang, J. and Wu, J. (2010) 'Co-extraction of egg white proteins using ion-exchange chromatography from ovomucinremoved egg whites', *Journal*

Of Chromatography B-Analytical Technologies In The Biomedical And Life Sciences, 878 (21), 1771-1776.

Osorio, M., Moreno, F. L., Raventós, M., Hernández, E. and Ruiz, Y. (2018) 'Progressive stirred freeze-concentration of ethanol-water solutions', *Journal of Food Engineering*, 224, 71-79.

Özişik, M. N. (1985) *Heat Exchanger. Heat Transfer: A Basic Approach Heat Exchanger*. New York: McGraw-Hill.

Pavon, J. A., Li, X., Chico, S., Kishnani, U., Soundararajan, S., Cheung, J., Li, H., Richardson, D., Shameem, M. and Yang, X. (2016) 'Analysis of monoclonal antibody oxidation by simple mixed mode chromatography', *Journal of Chromatography A*, 1431, 154-165.

Peng, D., Liao, F., Pan, Y., Chen, D., Liu, Z., Wang, Y. and Yuan, Z. (2016) 'Development a monoclonal antibody-based enzyme-linked immunosorbent assay for screening carotenoids in eggs', *Food Chemistry*, 202, 141-148.

Pereira, M. M., Cruz, R. A. P., Almeida, M. R., Lima, Á. S., Coutinho, J. A. P. and Freire, M. G. (2016) 'Single-Step Purification of Ovalbumin from Egg White Using Aqueous Biphasic Systems', *Process Biochemistry*, 51 (6), 781-791.

Perry, R. H. and Green, D. W. (1997) *Perry's Chemical Engineers' Handbook*. USA:McGraw-Hill.

Petzold, G., Orellana, P., Moreno, J., Cerda, E. and Parra, P. (2016) 'Vacuum-assisted block freeze concentration applied to wine', *Innovative Food Science & Emerging Technologies*, 36, 330-335.

Philips, D. C. (1966) 'The Three-dimensional Structure of an Enzyme Molecule', *Scientific American*, 215, 78-90.

Pishgar-Komleh, S. H., Keyhani, A., Msm, R. and Jafari, A. (2012) 'Application of response surface methodology for optimization of Picker-Husker harvesting losses in corn seed', *Iranica Journal of Energy and Environment*, 3 (2), 134-142.

Radosław Dembczyński, W. B., Krzysztof, R. and Jankowski, T. (2010) 'Lysozyme extraction from hen egg white in an aqueous two-phase system composed of ethylene oxide-propylene oxide thermoseparating copolymer and potassium phosphate', *Process Biochemistry*, 45, 369-374.

Raguraman, C. M., Ragupathy, A. and Sivakumar, L. (2013) 'Experimental Determination of Heat Transfer Coefficient in Stirred Vessel for Coal-Water Slurry Based on the Taguchi Method', *Journal of Engineering*, 2013, 8.

Ranade, V. V., Tayalia, Y. and Krishnan, H. (2002) 'CFD predictions of flow near impeller blades in baffled stirred vessels: Assessment of computational snapshot approach', *Chemical Engineering Communications*, 189 (7), 895-922.

Ratkje, S. K. and Flesland, O. (1995) 'Modelling the Freeze Concentration Process by Irreversible Thermodynamics', *Journal of Food Engineering*, 25 (4), 553-568.

Rayhan, F. A., Yanuar1 and Pamitran, A. S. (2018) Effect of initial seawater concentration on forming ice slurry for thermal energy storage in fishing vessel. *Proceedings of the 2018 E3S Web of Conferences*.

Rickard, D. L., Duncan, P. B. and Needham, D. (2010) 'Hydration potential of lysozyme: protein dehydration using a single microparticle technique', *Biophysical journal*, 98 (6), 1075-1084.

Robles, C. M., Quintanilla-Carvajal, M. X., Moreno, F. L., Hernández, E., Raventós, M. and Ruiz, Y. (2016) 'Ice morphology modification and solute recovery improvement by heating and annealing during block freeze-concentration of coffee extracts', *Journal of Food Engineering*, 189, 72-81.

Rodríguez, M., Luque, S., Alvarez, J. and Coca, J. (2000) 'A Comparative Study of Reverse Osmosis and Freeze Concentration for the Removal of Veleric Acid from Wastewaters', *Desalination*, 127 (1), 1-11.

Rosa, V. and Júnior, D. (2017) *Design of Heat Transfer Surfaces in Agitated Vessels*, in Murshed, S. M. S. & Lopes, M. M. (eds.) *Heat Exchanger: Design, Experiment and Simulation*. United Kingdom: InTech, pp. 37-60.

Ruby Figueroa, R. A., Cassano, A. and Drioli, E. (2011) 'Ultrafiltration of orange press liquor: Optimization for permeate flux and fouling index by response surface methodology', *Separation and Purification Technology*, 80 (1), 1-10.

Ruiz, R. Y. and Caicedo, L. A. (2009) Progressive Freeze Concentration of Sucrose Solutions. Proceedings of *The 8th World Congress of Chemical Engineering*. Canada.

Sabatkova, Z., Tokar, O. and Safarikova, M. (2007) 'Magnetic Cation Exchange Isolation of Lysozyme from Native Hen Egg White', *Food Technology and Biotechnology*, 45.

Sahasrabudhe, A. B., Desai, R. R. and Jabade, S. K. (2012) 'Modeling and Simulation of a Freeze Concentration Technique for Sugarcane Juice Concentration', *Applied Mechanics and Materials*, 110-116, 2768-2773.

Samsuri, S., Amran, N. A. and Jusoh, M. (2015) 'Spiral finned crystallizer for progressive freeze concentration process', *Chemical Engineering Research and Design*, 104, 280-286.

Samsuri, S. and Mohd Bakri, M. M. (2018) 'Optimization of fractional crystallization on crude biodiesel purification via response surface methodology', *Separation Science and Technology*, 53 (3), 567-572.

Sánchez, J., Ruiz, Y., Raventós, M., Auleda, J. M. and Hernández, E. (2010) 'Progressive freeze concentration of orange juice in a pilot plant falling film',. *Innovative Food Science & Emerging Technologies*, 11 (4), 644-651.

Sarafraz, M. M. and Hormozi, F. (2015) 'Intensification of forced convection heat transfer using biological nanofluid in a double-pipe heat exchanger', *Experimental Thermal and Fluid Science*, 66, 279-289.

Schimmelpennink, M. C., Vorselaars, A. D. M. and Grutters, J. C. (2019) *Chapter 19 - Biomarkers in Sarcoidosis*, in Baughman, R. P. & Valeyre, D. (eds.) *Sarcoidosis*. Philadelphia: Elsevier, pp. 219-238.

Schwerdtfeger, P. (1963) 'The Thermal Properties of Sea Ice', *Journal of Glaciology*, 4 (36), 789-807.

Sequera, S. C., Ruiz, Y., Moreno, F. L., Quintanilla-Carvajal, M. X. and Salcedo, F. (2019) 'Rheological evaluation of gelation during thermal treatments in block freeze concentration of coffee extrac', *Journal of Food Engineering*, 242, 76-83.

Shalaev, E., Soper, A., Zeitler, J. A., Ohtake, S., Roberts, C. J., Pikal, M. J., Wu, K. and Boldyreva, E. (2019) 'Freezing of Aqueous Solutions and Chemical Stability of Amorphous Pharmaceuticals: Water Clusters Hypothesis', *Journal of Pharmaceutical Sciences*, 108 (1), 36-49.

Sheikholeslami, M., Jafaryar, M., Farkhadnia, F., Gorji-Bandpy, M. and Ganji, D. (2014) 'Investigation of turbulent flow and heat transfer in an air to water double-pipe heat exchanger', *Neural Comput & Applic*, 26, 1-7.

Shin, H., Kalista, B., Jeong, S. and Jang, A. (2019) 'Optimization of simplified freeze desalination with surface scraped freeze crystallizer for producing irrigation water without seeding', *Desalination*, 452, 68-74.

Shirai, Y., Wakisaka, M., Miyawaki, O. and Sakashita, S. (1999) 'Effect of Seed Ice on Formation of Tube Ice with High Purity for a Freeze Wastewater Treatment System with a Bubble-Flow Circulator', *Water Resources Research*, 33 (5), 1325.

Silvetti, T., Morandi, S., Hintersteiner, M. and Brasca, M. (2017) *Chapter 22 - Use of Hen Egg White Lysozyme in the Food Industry*, in Hester, P. Y. (Ed.) *Egg Innovations and Strategies for Improvements*. San Diego: Academic Press, pp. 233-242.

Sirotkin, V. A. and Kuchierskaya, A. A. (2017) 'Lysozyme in water-acetonitrile mixtures: Preferential solvation at the inner edge of excess hydration', *The Journal of chemical physics*, 146 (21), 215101-215101.

Sofiah, H., Nora, A. and Marinah, M. A. (2010) 'The Influence of Polymer Concentration on Performance and Morphology of Asymmetric Ultrafiltration Membrane for Lysozyme Separation', *Journal of Applied Science*, 10, 3325-3330.

Stewart, M. and Lewis, O. T. (2013) *Chapter 1 - Heat Transfer Theory*, in Stewart, M. & Lewis, O. T. (eds.), *Heat Exchanger Equipment Field Manual*. Boston: Gulf Professional Publishing, pp. 1-91.

Sun, Y., Yang, G., Wen, C., Zhang, L. and Sun, Z. (2018) 'Artificial neural networks with response surface methodology for optimization of selective CO_2 hydrogenation using K-promoted iron catalyst in a microchannel reactor', *Journal of CO_2 Utilization*, 24, 10-21.

Tagaya, M., Nago, S., Matsuda, M., Takahashi, S., Okano, S. and Hara, K. (2017) 'Hemodialysis membrane coated with a polymer having a hydrophilic blood-contacting layer can enhance diffusional performance', *International Journal of Artificial Organs*, 40 (12), 665-669.

Tankrathok, A., Daduang, S., Patramanon, R., Arakai, T. and Thammasirirak, S. (2009) 'Purification Process for the Preparation and Characterization of Hen Egg White Ovalbumin, Lysozyme, Ovotransferrin, and Ovomucoid', *Preparative Biochemistry and Biotechnology*, 39, 380-399.

Ulrich, J. and Bülau, H. C. (2002) *Melt crystallization.*, in Myerson, A. S. (ed.), *Handbook of Industrial Crystallization (Second Edition)*. Woburn: Butterworth-Heinemann, pp. 161-179.

Ulrich, J. and Stelzer, T. (2019). *Melt Crystallization*, in Lee, A. Y., Myerson, A. S. & Erdemir, D. (eds.), *Handbook of Industrial Crystallization* (3 edition). Cambridge: Cambridge University Press, pp. 266-289.

van Putte, K. and Bakker, B. (1987) 'Crystallization Kinetics of Palm Oil', *Journal of The American Oil Chemists' Society*, 64 (8), 1138-1143.

Vellando, P., Fe, J., Juncosa, R. and Padilla, F. (2007) 'Improvements in Mixing Operations of Water Treatment Plants by Use of a Stable Finite Element Model', *Water environment research : a research publication of the Water Environment Federation*, 79, 625-640.

Wakisaka, M., Shirai, Y. and Sakashita, S. (2001) 'Ice crystallization in a pilot-scale freeze wastewater treatment system', *Chemical Engineering and Processing: Process Intensification*, 40 (3), 201-208.

Wang, J. and Wu, J. (2012) 'Effect of Operating Conditions on The Extraction of Ovomucin', *Process Biochemistry*, 47, 94-98.

Wang, Y., Lomakin, A., Latypov, R. F., Laubach, J. P., Hideshima, T., Richardson, P. G., Munshi, N. C., Anderson, K. C. and Benedek, G. B. (2013) 'Phase transitions in human IgG solutions', *The Journal of Chemical Physics*, 139 (12), 121904.

Ward, T. M., Edwards, R. A. and Tanner, R. D. (2007) 'Separating a Mixture of Egg Yolk and Egg White Using Foam Fractiona', *Applied Biochemistry and Biotechnology*, 137-140, 927-934.

Wu, J. and Acer-Lopez, A. (2012) 'Ovotransferrin: Structure, Bioactivities and Preparation', *Food Research International*, 46, 480-487.

Wu, Y.-Y., Xing, K., Zhang, X.-X., Wang, H., Wang, Y., Wang, F. and Li, J.-M. (2017) 'Influence of freeze concentration technique on aromatic and phenolic compounds, color attributes, and sensory properties of cabernet sauvignon wine', *Molecules*, 22, 1-18.

Wulandari, Z., Fardiaz, D., Budiman, C., Suryati, T. and Herawati, D. (2015) 'Purification of egg white lysozyme from Indonesian kampung chicken and ducks', *Media Peternakan Journal of Animal Science and Technology*, 38 (1), 18-26.

Yahya, N., Aziz, N. S., Nasir, M. Z., Zakaria, Z. Y., Ngadi, N. and Jusoh, M. (2019a) 'Heat transfer analysis on progressive freeze concentration of aqueous lysozyme solution', *Chemical Engineering Transactions*, 72, 133-138.

Yahya, N., Azlan, N., Amran, N. A., Zakaria, Z. Y., Ngadi, N., Hashim, R. and Jusoh, M. (2019b) 'Efficiency study on vertical-finned crystalliser for concentration of carrot juice', *Chemical Engineering Transactions*, 72, 355-360.

Yahya, N., Ismail, N., Zakaria, Z. Y., Ngadi, N., Abdul Rahman, R. and Jusoh, M. (2017a) 'The Effect of Coolant Temperature and Stirrer Speed for Concentration of Sugarcane via Progressive Freeze Concentration Process', *Chemical Engineering Transaction*, 56, 1147-1152.

Yahya, N., Jie, L. W., Zakaria, Z. Y., Ngadi, N., Mohamad, Z., Rahman, R. A. and Jusoh, M. (2017b).'Water purification of lake water using progressive freeze concentration method', *Chemical Engineering Transactions*, 56, 43-48.

Yan, E.-K., Zhao, F.-Z., Zhang, C.-Y., Yang, X.-Z., Shi, M., He, J., Liu, Y.-L., Liu, Y., Hou, H. and Yin, D.-C. (2018) 'Seeding Protein Crystallization with Cross-Linked Protein Crystals', *Crystal Growth & Design*, 18 (2), 1090-1100.

Yang, F., Tao, F., Li, C., Gao, L. and Yang, P. (2018) 'Self-assembled membrane composed of amyloid-like proteins for efficient size-selective molecular separation and dialysis', *Nature Communications*, 9 (1), 5443.

Yang, Y., Lu, Y., Guo, J. and Zhang, X. (2017) 'Application of freeze concentration for fluoride removal from water solution', *Journal of Water Process Engineering*, 19, 260-266.

Yeh, A. S. (2012) *Characterization of a novel weak cation-exchange hydrogel membrane through the separation of lysozyme from egg white*. Master of Applied Science, University of Waterloo, Ontario, Canada.

Yu, B. and Churchill, S. W. (2012) *Chapter One - Prediction of the Influence of Energetic Chemical Reactions on Forced Convective Heat Transfer*, in Sparrow, E. M., Cho, Y. I., Abraham, J. P. & Gorman, J. M. (eds.) *Advances in Heat Transfer*. Elsevier, 44, pp. 1-117):

Yu, T., Ma, J. and Zhang, L. Q. (2007) 'Factors Affecting Ice Crystal Purity During Freeze Concentration Process for Urine Treatment', *Journal of Harbin Institute of Technology*, 14 (5), 593-597.

Yu, X., Wang, J. and Ulrich, J. (2014) 'Purification of Lysozyme from Protein Mixtures by Solvent-Freeze-Out Technology', *Chemical Engineering and Technology*, 37 (8), 1353-1357.

Zambrano, A., Ruiz, Y., Hernández, E., Raventós, M. and Moreno, F. L. (2018) 'Freeze desalination by the integration of falling film and block freeze-concentration techniques', *Desalination*, 436, 56-62.

Zhang, B. (2016) *Chapter 6 - Impact Parameters and Deposition Rate*, in Zhang, B. (ed.) *Amorphous and Nano Alloys Electroless Depositions*. Oxford: Elsevier, pp. 323-381.

Zhang, P., Chen, G., Duan, J. and Wang, W. (2018) 'Mixing characteristics in a vessel equipped with cylindrical stirrer', *Results in Physics*, 10, 699-705.