UNIVERSITI PUTRA MALAYSIA

SUB-CRITICAL WATER TECHNOLOGY FOR ENHANCED EXTRACTION OF CARBOHYDRATES AND PROTEIN FROM* Chlorella vulgaris *MICROALGAE

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By

SAIDATUL ALIAA BINTI AWALUDDIN

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

February 2017

DEDICATION

This thesis is dedicated to my beloved parents, Awaluddin Bin Adnan and RohayatiBinti Tahir and family for all their endless love, patience, support and continuously pray for my success

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

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Algae have many advantages such as fast growth rate, non-arable land requirement for grow and contain substantial amount of biochemical compounds such as lipid, carbohydrate and protein for value added products production. One of the new key alternatives is to extract microalgal biochemical by utilizing subcritical water extraction (SWE). This technique applies hot water under pressure sufficiently retains water in liquid state at temperature below the supercritical values. This study investigates the effectiveness of SWE to extract protein and carbohydrate from microalgae, *Chlorella Vulgaris*. The study was divided into three parts; (i) investigation of various parameters that affect the biochemical production (protein and carbohydrates), (ii) optimization of SWE and (iii) pre-treatment validation. Four different parameters include temperature (180-374 ˚C), time (1-20min), biomass loading (5-40 wt%) and particle size of biomass (38-250μm) were used to investigate on microalgal SWE. The SWE process was further optimized using central composite design (CCD). It was found that the highest protein and carbohydrate concentration of 31.16 g/100 g and 14.2 g/100gwere obtained at 5 wt.%microalgal biomass with 90 μm particulate size treated at 277°C for 5 min. The TOC yield of 8.01g/100g was obtained at 229ºC for 3min with 10wt.%microalgal biomass at 75μm particle size. Based on the statistical analysis, microalgae loading and extraction temperature have shown the most significant factors that affect the overall extraction of protein whereas extraction temperature were the significant factors for carbohydrate production. TOC yields were only affected by the microalgal loading of the microalgal biomass. This study was further investigated the effect of pre-treatment prior to microalgal SWE. The biomass was irradiated under sonication as the pre-treatment technique with different process parameters. The results concluded that the pre-treatment was not required as the results were comparable with SWE without pre-treatment hence it gives advantages in reducing the extraction costs and avoiding environmental problems (e.g. usage of solvents).

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

TEKNOLOGI AIR SUB-KRITIKAL UNTUK MENINGKATKAN PENGEKSTRAKAN KARBOHIDRAT DAN PROTEIN DARI MIKROALGAL *Chlorella vulgaris*

Oleh

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Alga mempunyai banyak kelebihan seperti kadar pertumbuhan yang cepat, menggunakan tanah bukan pertanian untuk pertumbuhan dan mengandungi sejumlah besar sebatian biokimia seperti lipid, karbohidrat dan protein untuk nilai tambah pengeluaran produk. Salah satu alternatif baru adalah pengekstrakan protein dan karbohidrat daripada microalgal dengan menggunakan pengekstrakan air subkritikal (SWE). Kaedah ini menggunakan air panas dengan tekanan secukupnya bagi mengekalkan air dalam keadaan cecair pada suhu di bawah nilai superkritikal. SWE mempunyai beberapa kelebihan berbanding dengan teknik-teknik lain termasuklah ekstrak yang berkualiti tinggi, proses yang lebih cepat, mengurangkan penggunaan pelarut serta mengurangkan kos penggunaan ejen mengekstrak. Oleh itu, kajian ini mengkaji keberkesanan SWE untuk mengekstrak biomolekul dari mikroalga, *Chlorella vulgaris*. Kajian ini dibahagikan kepada tiga bahagian; (I) kajian ke atas pelbagai parameter yang memberi kesan kepada produksi biokimia (protein dan karbohidrat), (ii) pengoptimuman SWE dan (iii) pengesahan pra-rawatan. Empat parameter yang berbeza digunakan dalam proses ini adalah termasuk suhu (180-374 ˚C), masa (1-20min), pemuatan biojisim (5-40 wt%) dan saiz zarah biojisim (38-250µm) yang digunakan untuk mengkaji proses SWE menggunakan microalgal. Proses SWE dioptimumkan dengan menggunakan Reka bentuk Komposit Pusat (CCD).Berdasarkan kajian yang dijalankan, kepekatan protein dan karbohidrat yang paling tinggi dapat diekstrak adalah 31.16 g / 100 g dan 14.2 g / 100g dan telah diekstrak pada suhu 277 ° C selama 5 min menggunakan mikroalga bersaiz 90 µm dan jumlah pemuatan biojisim ialah 5 wt%. TOC dapat diekstrak sebanyak 8.01g / 100g pada suhu 229ºC selama 3 minit dengan jumlah pemuatan biojisim sebanyak 10wt.% dan saiz zarah mikroalga ialah 75µm. Berdasarkan analisis statistik yang dijalankan, pemuatan mikroalga dan suhu pengekstrakan telah menunjukkan faktor yang paling penting yang mempengaruhi keseluruhan pengekstrakan protein, manakala pemuatan mikroalga dan masa pengekstrakan merupakan parameter paling penting bagi pengekstrakan karbohidrat. Pengekstrakan TOC hanya dipengaruhi oleh pemuatan microalgal.Selain itu, kesan terhadap pra-rawatan sebelum microalgal SWE telah dikaji. Mikroalgal telah diradiasi

menggunakan teknik sonication sebagai teknik pra rawatan dengan menggunakan proses parameter yang berbeza-beza. Berdasarkan hasil eksperimen tersebut, dapat disimpulkan bahawa pra-rawatan mikroalgal tidak diperlukan kerana hasilnya sama dengan SWE tanpa pra-rawatan. Oleh itu, ini dapat mengurangkan kos pengekstrakan dan juga dapat mengelakkan daripada masalah alam sekitar (contohnya penggunaan pelarut).

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

SWE	Subcritical water extraction
CCD	Central composite design
TOC	Total organic carbon
SEM	Scanning Electron Microscopy
FTIR	Fourier TransfromInFra-Red
HPLC	High Performance Liquid Chromatography
PBR	Photobioreactor
SFE	Supercritical fluid extraction
CHNS	Carbon, Hydrogen, Nitrogen, Sulphur
EDS	Energy dispersive
BSA	Bovine Serum Albumin
UV	Ultraviolet
MW	Molecular weight
IC	Inorganic carbon
TC	Total carbon

CHAPTER 1

INTRODUCTION

1.1 Background of study

The increasing of world population creates various environmental problems and demands on energy supply. It is important to develop an alternative and more sustainable energy source based on renewable resources. Biofuel such as biodiesel and bioethanol are viable alternative energy sources to alleviate the existing dependence on petroleum-based fuel (Lang et al., 2001). These types of fuels are produced from biomass. Biomass is bioorganic-based matter which derived from waste produced from living organisms such as agricultural, industrial, plant and etc. (Lucia et al., 2006).

In recent years, there have been an increasing interest to produce biofuel from microalgae due to its advantage of high growth rate (double their size in 24 hours), easy to grow, efficient carbon dioxide fixation and does not required arable land to growth (C. Y. Chen et al., 2013). It contains high protein, lipids and carbohydrates compositions that can be further converted to produce variety of bio-based products (Chisti, 2007; Sheehan et al, 1998). For instance, the protein content in microalgae can be used for animal feed production, lipids for biodiesel and carbohydrate for ethanol production (C. Y. Chen et al., 2013). It also has potential to be used as a source of natural resource of different functional compounds such as Omega-3, chlorophyll and etc(M. Plaza et al., 2010). Some of microalgal species is capable in treating wastewater and the organic and inorganic nutrients in wastewater can further utilize as growth nutrients for microalgae. This can make the process in producing bioproducts from microalgae more viable, cost-effective and sustainable.

Chemical composition of each microalgae strain is not similarly constant, but varies depending on various environmental parameters such as temperature, illumination, pH, mineral, CO_2 supply and mixing velocity (W. Becker, 2004). According to Becker, 2004, variations in culture conditions, or changing of physical parameters such as radiation intensity, population density, light/ dark growth, producing different microalgal compositions. Biomolecule compounds are also present in microalgal cells and synthesized as secondary metabolites via specific metabolic pathways (Morais et al., 2015). Microalgal biomolecule metabolites are intracellularly produced and entrapped within the cells, thus an effective extraction technology is required to release those biomolecule products (Morais et al., 2015).

One of the emerging extraction techniques is using subcritical water extraction (SWE) technology. There has been an increasing interest in extraction of compounds from biomass materials using SWE (Macías-Sánchez et al., 2007). SWE utilizes water at high temperature ranging between 100°C (boiling point) to 374°C (critical point) and pressure at high enough to keep it in the liquid state (Singh &Saldaña, 2011). Two important characteristics during SWE are water ionization and dielectric potential. As the temperature of water elevated, its hydrogen bonding cleaves with decreasing dielectric constant and polarity (Mazaheri et al, 2010). This resulted in the increment of the hydrogen ion concentrations (Abdelmoez & Yoshida, 2006). Using water as an extraction solvent offers lower production cost, safety, no net of pollution and non-toxic. Other than that, this technology is an attractive method due to shorter production period and milder operating conditions compared to the existing conventional methods. As the technology is scarcely reported to extract products from microalgae, therefore this work investigates different process conditions include temperature, extraction time and biomass loading to extract protein and carbohydratescompounds from microalgal biomass.

1.2 Problem statement

Limited petroleum resources and environmental pollution are the two critical problems nowadays. Increasing amounts of fossil utilization is the reason of global warming and climate change. To solve this problem, developing renewable energy alternatives using biofuel have triggered researcher interest. The first and second generation feedstock are not favorable for alternative energy resources due to many limitations. First generation feedstock which are derived from sources like starch, sugar, animal fats, vegetable oil and etc. creates 'food vs fuel' issue. They need huge arable land for cultivation hence not efficient for energy demands. Meanwhile, the second generation of biofuel feedstock are derived from on-edible crops, lignocellulosic biomass, or woody crops. They are also not favorable for biofuel production due to the presence of lignin thus require pre-treatment steps. This pretreatment cost makes the overall process to produce biofuel/bioproducts is not efficient.

Microalgae have been considered as third generation biofuel. It is a sustainable and renewable biomass for the production of biofuel and other bioproducts. Microalgae can be cultured and harvested according to the needs of the current process (i.e. high yield of omega-3 and lipid content) and they can also be genetically manipulated to produce high biomass yields. It contains three main components; lipids, carbohydrate and protein. Lipid is used for biofuel and pharmaceutical products (e.g. Omega-3 oil). Carbohydrate in microalgae can be found in the form of starch, glucose, sugars and other polysaccharides (Ördög et al., 2004). Recently, carbohydrates from microalgae is considered as a promising and inexpensive feedstock for biofuel production (Zhao et al., 2013). The most widely used biofuel is bioethanol, which produces from fermenting sugar-based or starch-based feedstock (C. Y. Chen et al., 2013).It also could be converted to produce fine chemicals such as ethanol and acetic acid. Furthermore, protein from microalgae could be converted to produce many commodities such as food, animal feed and health products.

Choosing the right biomass feedstock and extraction method are the important factors to produce alternative energy source that will reduce environmental pollution, lesser time and cost production. One of the new key options is to extract lipids from microalgal biomass using a sub-critical water technology (SWE). The technology provides lower production cost, greater reduction of solvents using a non-toxic green solvent (water), milder operating conditions, and a shorter production period compared to other conventional methods such as chemical and biological. Due to the fact that sub-critical water extraction method is a solvent free extraction, the current investigation aims to optimize the parameters that work best in extracting protein and carbohydrate compounds from microalgae. The works that are mainly done by others related to this field tend to focus more on maximizing the lipid extraction but only few have done on maximizing carbohydrate and protein compounds.

1.3 Research question

1. How is the subcritical water extraction (SWE) different from other extraction technologies (in term of production of carbohydrate and protein compounds)?
2. What are the key parameters to be optimized in the extraction process to increase the carbohydrate and protein compounds yields?
3. How do SWE affect the characteristic of carbohydrate and protein compounds from microalgae?
4. What are the significant variables that will affect the SWE of carbohydrate and protein compounds?

1.4 Objective

The main objective of this research is to investigate the capability of SWE in extracting carbohydrates and protein compounds from *C. vulgaris* biomass. The objectives are divided into three sub-objective as follow:

1. To investigate the factors that affect the extraction process of microalgae via subcritical water extraction technique.
2. To analyze and characterize the extracted carbohydrates and protein of *C. vulgaris* biomass.
3. To optimize the SWE process using Central Composite Design (CCD) approachfor high yield of carbohydrates and protein production from *C. vulgaris* biomass.

1.5 Scope of study

The purpose of this study is to extract compounds of *C. vulgaris* via subcritical water extraction (SWE). The study is divided into three main stages (1) investigation of different parameters that affect on the extraction process, (2) characterization of the extracted products and (3) optimization of process parameters for high products yields.

1.5.1 Investigation of different parameters on SWE of *C.vulgaris.*

The main objective of this research is to investigate the effect of different parameters in extracting carbohydrate and protein compounds from *C. vulgaris* using SWE. The parameters include temperature (180 - 374 °C), extraction time (1 - 20 min), biomass particulate size (38 - 250 µm) and microalgal biomass loading (5 - 40 %).

1.5.2 Characterization of the extracted products

The characterization of *C. vulgaris* biomass covers four different types of analysis include proximate, ultimate, Scanning Electron Microscopy (SEM) and Fourier TransfromInFra-Red (FTIR). The characterization of the extracted products includes the analysis of carbohydrate, protein and Total organic compounds (TOC) concentration in the *C. vulgaris.* Amino acids profile and sugar profile was characterized using High Performance Liquid Chromatography (HPLC).

1.5.3 Optimization of process parameters

Central composite design (CCD) approach is used to design the optimization study of *C. vulgaris* biomass extraction. The optimization analysis using *Statistica* software is used to obtain relationship between the investigated parameters with the products. The optimized parameters resulting high carbohydrate and protein compounds production from the extraction of *C. vulgaris* biomass will ensure a right direction towards the achievement to use biofuel for different applications. Based on the optimization findings, the kinetic study is performed to evaluate the extraction rate as the function of temperature. The experimental results are analyzed using the second order model and the activation energy is calculated using Arrhenius equation.

REFERENCES

Abdelmoez, W., & Yoshida, H. (2006). Simulation of fast reactions in batch reactors under sub-critical water condition. *AIChE Journal*, *52*(10), 3600–3611.

Ahmadian-Kouchaksaraie, Z., Niazmand, R., & Najafi, M. N. (2016). Optimization of the subcritical water extraction of phenolic antioxidants from Crocus sativus petals of saffron industry residues: Box-Behnken design and principal component analysis. *Innovative Food Science and Emerging Technologies*, *36*, 234–244.

Alonso, D. M., Bond, J. Q., & Dumesic, J. a. (2010). Catalytic conversion of biomass to biofuels. *Green Chemistry*, *12*(9), 1493.

Alupului, A., Calinescu, I., & Lavric, V. (2006). Ultrasonic vs. Microwave Extraction Intensification of Active Principles from Medicinal Plants.

Anastasakis, K., & Ross, A. B. (2011). Hydrothermal liquefaction of the brown macro-alga Laminaria Saccharina: Effect of reaction conditions on product distribution and composition. *Bioresource Technology*, *102*(7), 4876–4883.

Antizar-Ladislao, B., & Turrion-Gomez, J. L. (2008). Second-generation biofuels and local bioenergy systems. *Biofuels, Bioproducts and Biorefining*, *2*, 455–469.

Araujo, G. S., Matos, L. J. B. L., Fernandes, J. O., Cartaxo, S. J. M., Gonçalves, L. R. B., Fernandes, F. A. N., & Farias, W. R. L. (2013). Extraction of lipids from microalgae by ultrasound application: Prospection of the optimal extraction method. *Ultrasonics Sonochemistry*, *20*(1), 95–98.

Aresta, M., Dibenedetto, A., Carone, M., Colonna, T., & Fragale, C. (2005). Production of biodiesel from macroalgae by supercritical CO2 extraction and thermochemical liquefaction. *Environmental Chemistry Letters*, *3*(3), 136–139.

Asl, A. H., & Khajenoori, M. (2013). Subcritical Water Extraction. In *Mass Transfer* (pp. 459–487).

Barbarino, E., & Lourenço, S. O. (2005). An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. *Journal of Applied Phycology*, *17*(5), 447–460.

Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, *25*(2), 207–210.

Becker, W. (2004). Microalgae in human and animal nutrition. *Handbook of Microalgal Culture*, 312–351.

Bi, Z., & He, B. B. (2013). Characterization of Microalgae for the Purpose of Biofuel Production. *Transactions of the ASABE*, *56*(4), 1529–1539.

Borowitzka, M. A. (1995). Microalgae as sources of pharmaceuticals and other biologically active compounds. *Journal of Applied Phycology*, *7*, 3–15.

Bright, R. M., Strømman, A. H., & Hawkins, T. R. (2010). Environmental assessment of wood-based biofuel production and consumption scenarios in Norway. *Journal of Industrial Ecology*, *14*(3), 422–439.

Canela, A. P. R. F., Rosa, P. T. V, Marques, M. O. M., & Meireles, M. A. a. (2002). Supercritical Fluid Extraction of Fatty Acids and Carotenoids from the Microalgae Spirulina maxima. *Industrial & Engineering Chemistry Research*, *41*(12), 3012–3018.

Carriquiry, M. A., Du, X., & Timilsina, G. R. (2011). Second generation biofuels: Economics and policies. *Energy Policy*, *39*(7), 4222–4234.

Cechinel, M. A. P., Mayer, D. A., Pozdniakova, T. A., Mazur, L. P., Boaventura, R. A. R., de Souza, A. A. U., &Vilar, V. J. P. (2016). Removal of metal ions from a petrochemical wastewater using brown macro-algae as natural cation-exchangers. *Chemical Engineering Journal*, *286*, 1–15.

Chakraborty, M., Miao, C., McDonald, A., & Chen, S. (2012). Concomitant extraction of bio-oil and value added polysaccharides from Chlorella sorokiniana using a unique sequential hydrothermal extraction technology. *Fuel*, *95*, 63–70.

Chen, C. Y., Zhao, X. Q., Yen, H. W., Ho, S. H., Cheng, C. L., Lee, D. J., & Chang, J. S. (2013). Microalgae-based carbohydrates for biofuel production. *Biochemical Engineering Journal*, *78*, 1–10.

Chen, P., Min, M., Chen, Y., Wang, L., Li, Y., Chen, Q., & Ruan, R. (2009). Review of the biological and engineering aspects of algae to fuels approach. *International Journal of Agricultural and Biological Engineering*, *2*(4), 1–30.

Chisti, Y. (2007). Biodiesel from microalgae. *Trends in Biotechnology*, *25*, 294–306.

Davis, P. J., & Williams, S. C. (1998). Protein modification by thermal processing. *Allergy*, *53*(46), 102–105.

Davis, T. A., Volesky, B., & Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research*, *37*(18), 4311–4330.

De Vries, S. C., van de Ven, G. W. J., van Ittersum, M. K., & Giller, K. E. (2010). Resource use efficiency and environmental performance of nine major biofuel crops, processed by first-generation conversion techniques. *Biomass and Bioenergy*, *34*(5), 588–601.

Dragone, G., Fernandes, B., Vicente, A., & Teixeira, J. (2010). Third generation biofuels from microalgae. In *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*. (p.p 1355–1366).

Fatih Demirbas, M. (2009). Biorefineries for biofuel upgrading: A critical review. *Applied Energy*, *86* (1), 151–161.

Field, L. M., Fagerberg, W. R., Gatto, K. K., & Böttger, S. A. (2016). A comparison of protein extraction methods optimizing high protein yields from marine algae and cyanobacteria. *Journal of Applied Phycology*, 1–8.

Gallagher, W. (1997). FTIR Analysis of Protein Structure. *Biochemistry*, *18*, 662–666.

Garcia-Moscoso, J. L., Obeid, W., Kumar, S., & Hatcher, P. G. (2013). Flash hydrolysis of microalgae (*Scenedesmus* sp.) for protein extraction and production of biofuels intermediates. *Journal of Supercritical Fluids*, *82*, 183–190.

Garlick, P. J. (2005). The Role of Leucine in the Regulation of Protein Metabolism. *J Nutr*, 1553–1556.

Giordano, M., Heraud, P., Beardall, J., Wood, B., & McNaughton, D. (2001). Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalga chaetoceros muellerii (bacillariophyceae). *J. Phycol*, *37*, 271–279.

Glithero, N. J., Wilson, P., & Ramsden, S. J. (2013). Straw use and availability for second generation biofuels inEngland. *Biomass and Bioenergy*, *55*(2013), 311–321.

Graboski, M. S., & McCormick, R. L. (1998). Combustion of fat and vegetable oil derived fuels in diesel engines. *Progress in Energy and Combustion Science*, *24*(2), 125–164.

Haghighat Khajavi, S., Kimura, Y., Oomori, T., Matsuno, R., & Adachi, S. (2005). Kinetics on sucrose decomposition in subcritical water. *LWT - Food Science and Technology*, *38*(3), 297–302.

Halim, R., Gladman, B., Danquah, M. K., & Webley, P. a. (2011). Oil extraction from microalgae for biodiesel production. *Bioresource Technology*, *102*(1), 178–185.

Harun, R., Jason, W. S. Y., Cherrington, T., & Danquah, M. K. (2011). Exploring alkaline pre-treatment of microalgal biomass for bioethanol production. *Applied Energy*, *88*(10), 3464–3467.

Harun, R., Singh, M., Forde, G. M., & Danquah, M. K. (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews*, *14*(3), 1037–1047.

Hassas-Roudsari, M. (2007). *Subcritical water extraction of antioxidant compounds from canola meal*. University of Saskatchewan.

Hassas-Roudsari, M., Chang, P., Pegg, R., & Tyler, R. (2009). Antioxidant capacity of bioactives extracted from canola meal by subcritical water, ethanolic and hot water extraction. *Food Chemistry*, *114*(2), 717–726.

Havlík, P., Schneider, U. A., Schmid, E., Böttcher, H., Fritz, S., Skalský, R., & Obersteiner, M. (2011). Global land-use implications of first and second generation biofuel targets. *Energy Policy*, *39*(10), 5690–5702.

Hedrick, J. L., Mulcahey, L. J., & Taylor, L. T. (1992). Supercritical fluid extraction. *Mikrochimica Acta*, *108*, 115–132.

Helm, R. M., & Burks, A. W. (1996). Hypoallergenicity of rice protein. *Cereal foods world*, *41*(11), 839–843.

Herrero, M., Ibáñez, E., Señoráns, J., & Cifuentes, A. (2004). Pressurized liquid extracts from Spirulina platensis microalga. *Journal of Chromatography A*, *1047*(2), 195–203.

Herrero, M., Martin-Alvarez, P. J., Senorans, F. J., Cifuentes, A., & Ibanez, E. (2005). Optimization of accelerated solvent extraction of antioxidants from Spirulina platensis microalga. *Food Chemistry*, *93*(3), 417–423.

Hickey, R. M. (2012). Extraction and Characterization of Bioactive Carbohydrates with Health Benefi ts from Marine Resources: Macro- and Microalgae, Cyanobacteria, and Invertebrates. In *Marine Bioactive Compounds: Sources, Characterization and Applications* (pp. 159–172).

Huang, Y., Chen, Y., Xie, J., Liu, H., Yin, X., & Wu, C. (2016). Bio-oil production from hydrothermal liquefaction of high-protein high-ash microalgae including wild Cyanobacteria sp. and cultivated Bacillariophyta sp. *Fuel*, *183*, 9–19.

Jiménez-Carmona, M. M., & Luque de Castro, M. D. (1999). Isolation of eucalyptus essential oil for GC-MS analysis by extraction with subcritical water. *Chromatographia*, *50*(9-10), 578–582.

John, R. P., Anisha, G. S., Nampoothiri, K. M., & Pandey, A. (2011). Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresource Technology*, *102*(1), 186–193.

Jung, K. W., Jeong, T. U., Kang, H. J., & Ahn, K. H. (2016). Characteristics of biochar derived from marine macroalgae and fabrication of granular biochar by entrapment in calcium-alginate beads for phosphate removal from aqueous solution. *Bioresource Technology*, *211*, 108–116.

Karmee, S. K. (2016). Liquid biofuels from food waste: Current trends, prospect and limitation. *Renewable and Sustainable Energy Reviews*, *53*, 945–953.

Karthik, R., & Meenakshi, S. (2014). Facile synthesis of cross linked-chitosan-grafted-polyaniline composite and its Cr(VI) uptake studies. *International Journal of Biological Macromolecules*, *67*, 210–219.

Katsanos, C. S., Kobayashi, H., Sheffield-Moore, M., Aarsland, A., & Wolfe, R. R. (2006). A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *American Journal of Physiology. Endocrinology and Metabolism*, *291*(2), 381–387.

Khajavi, S. H., Kimura, Y., Oomori, T., Matsuno, R., & Adachi, S. (2005). Degradation kinetics of monosaccharides in subcritical water. *Journal of Food Engineering*, *68*(3), 309–313.

Khan, J. K., Kuo, Y. H., Kebede, N., & Lambein, F. (1994). Determination of non-protein amino acids and toxins in Lathyrus by high-performance liquid chromatography with precolumn phenyl isothiocyanate derivatization. *Journal of Chromatography A*, *687*(1), 113–119.

Khuwijitjaru, P., Pokpong, A., Klinchongkon, K., & Adachi, S. (2014). Production of oligosaccharides from coconut meal by subcritical water treatment. *International Journal of Food Science & Technology*, *49*(8), 1946–1952.

Ko, M.-J., Cheigh, C.-I., Cho, S.-W., & Chung, M.-S. (2011). Subcritical water extraction of flavonol quercetin from onion skin. *Journal of Food Engineering*, *102*(4), 327–333.

Koberg, M., Cohen, M., Ben-Amotz, A., & Gedanken, A. (2011). Bio-diesel production directly from the microalgae biomass of Nannochloropsis by microwave and ultrasound radiation. *Bioresource Technology*, *102*(5), 4265–4269.

Kong, J., & Yu, S. (2007). Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structures. *Acta Biochimica et Biophysica Sinica*, *39*(8), 549–559.

Koomyart, I., Nagamizu, H., Khuwijitjaru, P., Kobayashi, T., Shiga, H., Yoshii, H., & Adachi, S. (2014). Subcritical Water Treatment for Producing Seasoning From Semidried Isada Krill. *Journal of Food Process Engineering*, 1–49.

Kruse, a., & Dinjus, E. (2007). Hot compressed water as reaction medium and reactant. 2. Degradation reactions. *Journal of Supercritical Fluids*, *41*(3), 361–379.

Kumari, P., Reddy, C. R. K., & Jha, B. (2011). Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. *Analytical Biochemistry*, *415*(2), 134–144.

Lal, R. (2005). World crop residues production and implications of its use as a biofuel. *Environment International*, *31*(4), 575–584.

Landero, J. L., Beltranena, E., Cervantes, M., Araiza, A. B., & Zijlstra, R. T. (2012). The effect of feeding expeller-pressed canola meal on growth performance and diet nutrient digestibility in weaned pigs. *Animal Feed Science and Technology*, *171*(2012), 240–245.

Li, F., Yin, Y., Tan, B., Kong, X., & Wu, G. (2011). Leucine nutrition in animals and humans: MTOR signaling and beyond. *Amino Acids*, *41*(5), 1185–1193.

Liang, X., & Fan, Q. (2013). Application of Sub-Critical Water Extraction in Pharmaceutical Industry. *Journal of Materials Science and Chemical Engineering*, *5*(1), 1–6.

Lowry, O. H., Rosebrough, N. J., Randall, R. J., & Lewis Farr, A. (1951). Protein Measurement with The Folin Phenol Reagent. *Journal of Biological Chemistry*, *193*(1), 265–275.

Lucia, L. A., Argyropoulos, D. S., Adamopoulos, L., & Gaspar, A. R. (2006).

Chemicals and energy from biomass. *Canadian Journal of Chemistry*, *84*, 960–970.

Luo, X., Duan, Y., Yang, W., Zhang, H., Li, C., & Zhang, J. (2017). Structural elucidation and immunostimulatory activity of polysaccharide isolated by subcritical water extraction from Cordyceps militaris. *Carbohydrate Polymers*, *157*, 794–802.

Macías-Sánchez, M. D., Mantell, C., Rodríguez, M., Martínez De La Ossa, E., Lubián, L. M., & Montero, O. (2005). Supercritical fluid extraction of carotenoids and chlorophyll a from Nannochloropsis gaditana. *Journal of Food Engineering*, *66*(2), 245–251.

Macías-Sánchez, M. D., Mantell, C., Rodríguez, M., Martínez de la Ossa, E., Lubián, L. M., & Montero, O. (2007). Supercritical fluid extraction of carotenoids and chlorophyll a from Synechococcus sp. *The Journal of Supercritical Fluids*, *39*(3), 323–329.

Maehre, H. K., Malde, M. K., Eilertsen, K.-E., & Elvevoll, E. O. (2014). Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. *Journal of the Science of Food and Agriculture*, *94*(15), 3281–3290.

Maity, J. P., Bundschuh, J., Chen, C.-Y., & Bhattacharya, P. (2014). Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. *Energy*, *78*, 104–113.

Marcet, I., Álvarez, C., Paredes, B., & Díaz, M. (2016). The use of sub-critical water hydrolysis for the recovery of peptides and free amino acids from food processing wastes. Review of sources and main parameters. *Waste Management*, *49*, 364–371.

Markou, G., Angelidaki, I., & Georgakakis, D. (2012). Microalgal carbohydrates: An overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. *Applied Microbiology and Biotechnology*, *96*(3), 631–645.

Mazaheri, H., Lee, K. T., Bhatia, S., & Mohamed, A. R. (2010). Subcritical water liquefaction of oil palm fruit press fiber in the presence of sodium hydroxide: An optimisation study using response surface methodology. *Bioresource Technology*, *101*(23), 9335–9341.

Melis, A., & Happe, T. (2001). Hydrogen Production . Green Algae as a Source of Energy. *Plant Physiology*, *127*, 740–748.

Michalak, I., Dmytryk, A., Wieczorek, P. P., Rój, E., Bogus, B., Górka, B. B., & Chojnacka, K. (2015). Supercritical Algal Extracts : A Source of Biologically Active Compounds from Nature. *Journal of Chemistry*, 1–15.

Mierke-Klemeyer, S., Larsen, R., Oehlenschläger, J., Maehre, H., Elvevoll, E. O., Bandarra, N. M., & Luten, J. (2008). Retention of health-related beneficial components during household preparation of selenium-enriched African catfish (Clarias gariepinus) fillets. *European Food Research and Technology*, *227*(3), 827–833.

Mobin, S., & Alam, F. (2014). Biofuel Production from Algae Utilizing Wastewater. In *19th Australasian Fluid Mechanics Conference*. Melbourne, Australia.

Mohr, A., & Raman, S. (2013). Lessons from first generation biofuels and implications for the sustainability appraisal of second generation biofuels. *Energy Policy*, *63*, 114–122.

Morais, M. G. De, Vaz, S., Morais, E. G. De, Alberto, J., & Costa, V. (2015). Biologically Active Metabolites Synthesized by Microalgae. *BioMed Research International*, *2015*, 1–15.

Mortimer, M., & Taylor, P. (2002). *Chemical kinetics and mechanisms*. UK: Royal society of chemistry.

Muangrat, R., Onwudili, J. a., & Williams, P. T. (2010). Influence of alkali catalysts on the production of hydrogen-rich gas from the hydrothermal gasification of food processing waste. *Applied Catalysis B: Environmental*, *100*(3-4), 440–449.

Müller-langer, F., Majer, S., & Keeffe, S. O. (2014). Benchmarking biofuels — a comparison of technical , economic and environmental indicators. *Energy, Sustainability and Society*, *20*(4), 1–14.

Munir, N., Sharif, N., Naz, S., & Saleem, F. (2013). Harvesting and Processing of Microalgae Biomass Fractions for Biodiesel Production (a Review). *Sci., Tech. and Dev.*, *32*(3), 235–243.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis*, *41*(5), 1523–1542.

Nahar, L., & Sarker, S. D. (2005). Supercritical Fluid Extraction. *Natural Products Isolation*, *20*, 47–76.

Naik, S. N., Goud, V. V., Rout, P. K., & Dalai, A. K. (2010). Production of first and second generation biofuels: A comprehensive review. *Renewable and Sustainable Energy Reviews*, *14*(2), 578–597.

Ndlela, S. C., De Moura, J. M. L. N., Olson, N. K., & Johnson, L. A. (2012). Aqueous extraction of oil and protein from soybeans with subcritical water. *JAOCS, Journal of the American Oil Chemists' Society*, *89*(6), 1145–1153.

Nielsen, S. S. (2010). Food Analysis Laboratory Manual. In *Food Science Texts series* (pp. 47–53). doi:10.1007/978-1-4419-1463-7

Nurjanah, Nurilmala, M., Hidayat, T., & Sudirdjo, F. (2016). Characteristics of Seaweed as Raw Materials for Cosmetics. *Aquatic Procedia*, *7*, 177–180.

Ördög, V., Stirk, W. A., Lenobel, R., Bancířová, M., Strnad, M., Van Staden, J., & Németh, L. (2004). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*, *16*(4), 309–314.

Palavra, a. M. F., Coelho, J. P., Barroso, J. G., Rauter, a. P., Fareleira, J. M. N. a, Mainar, a., & Novais, J. M. (2011). Supercritical carbon dioxide extraction of bioactive compounds from microalgae and volatile oils from aromatic plants. *Journal of Supercritical Fluids*, *60*, 21–27.

Pavlić, B., Vidović, S., Vladić, J., Radosavljević, R., Cindrić, M., & Zeković, Z. (2016). Subcritical water extraction of sage (Salvia officinalis L.) by-products − process optimization by response surface methodology. *The Journal of Supercritical Fluids*, *116*, 36–45.

Pechsiri, J. S., Thomas, J.-B. E., Risén, E., Ribeiro, M. S., Malmström, M. E., Nylund, G. M.,& Gröndahl, F. (2016). Energy performance and greenhouse gas emissions of kelp cultivation for biogas and fertilizer recovery in Sweden. *Science of The Total Environment*, *573*, 347–355.

Plaza, M., Amigo-Benavent, M., del Castillo, M. D., Ibáñez, E., & Herrero, M. (2010). Facts about the formation of new antioxidants in natural samples after subcritical water extraction. *Food Research International*, *43*(10), 2341–2348.

Plaza, M., Santoyo, S., Jaime, L., García-Blairsy Reina, G., Herrero, M., Señoráns, F. J., & Ibáñez, E. (2010). Screening for bioactive compounds from algae. *Journal of Pharmaceutical and Biomedical Analysis*, *51*(2), 450–455.

Popoola, T. O. S., & Yangomodou, O. D. (2006). Extraction properties and utilization potentials of cassava seed oil. *Biotechnology*, *5*(1), 38–41.

Pourali, O., Asghari, F. S., & Yoshida, H. (2009). Sub-critical water treatment of rice bran to produce valuable materials. *Food Chemistry*, *115*(1), 1–7.

Pradhan, R. C., Mishra, S., Naik, S. N., Bhatnagar, N., & Vijay, V. K. (2011). Oil expression from Jatropha seeds using a screw press expeller. *Biosystems Engineering*, *109*(2), 158–166.

Reddy, H. K., Muppaneni, T., Sun, Y., Li, Y., Ponnusamy, S., Patil, P. D., & Deng, S. (2014). Subcritical water extraction of lipids from wet algae for biodiesel production. *Fuel*, *133*, 73–81.

Rennie, M. J., Bohe, J., & Wolfe, R. R. (2002). Latency, duration and dose response relationships of amino acid effects on human muscle protein synthesis. *The Journal of Nutrition*, *132*(10), 3225S–7S.

Rivera, A., Bell, E. F., & Bier, D. M. (1993). Effect of intravenous amino acids on protein metabolism of preterm infants during the first three days of life. *Pediatric Research*, *33*(2), 106–111.

Rodríguez-Meizoso, I., Jaime, L., Santoyo, S., Señoráns, F. J., Cifuentes, a., & Ibáñez, E. (2010). Subcritical water extraction and characterization of bioactive compounds from Haematococcus pluvialis microalga. *Journal of Pharmaceutical and Biomedical Analysis*, *51*(2), 456–463.

Ross, A. B., Jones, J. M., Kubacki, M. L., & Bridgeman, T. (2008). Classification of macroalgae as fuel and its thermochemical behaviour. *Bioresource Technology*, *99*(14), 6494–6504.

Rovio, S., Hartonen, K., Holm, Y., Hiltunen, R., & Riekkola, M. (1999). Extraction of clove using pressurized hot water.*Flavour and Fragrance Journal*, *404*, 399–404.

Sabiha Hanim, S., Norsyabilah, R., Nor Suhaila, M. H., Noraishah, A., & Siti Kartina, A. K. (2012). Effects of temperature, time and pressure on the hemicelluloses yield extracted using subcritical water extraction. *Procedia Engineering*, *42*(2012), 562–565.

Safi, C., Charton, M., Pignolet, O., Silvestre, F., Vaca-Garcia, C., & Pontalier, P. Y. (2013). Influence of microalgae cell wall characteristics on protein extractability and determination of nitrogen-to-protein conversion factors. *Journal of Applied Phycology*, *25*(2), 523–529.

Salak, F., Pourali, O., & Yoshida, H. (2009). Application of Subcritical Water Treatment for Production of Phenolic Compounds from Rice Bran Biomass. In *Proceedings of the World Congress on Engineering and Computer Science 2009* (Vol. I, pp. 6–8).

Sarkar, S., & Science, N. (2013). Extraction of Carbohydrates and Phenolics from Barley Hull using Pressurized / Subcritical Fluids.

Sereewatthanawut, I., Prapintip, S., Watchiraruji, K., Goto, M., Sasaki, M., & Shotipruk, A. (2008). Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. *Bioresource Technology*, *99*(3), 555–61.

Shaddel, R., Maskooki, A., Haddad-Khodaparast, M. H., Azadmard-Damirchi, S., Mohamadi, M., & Fathi-Achachlouei, B. (2014). Optimization of extraction process of bioactive compounds from Bene hull using subcritical water. *Food Science and Biotechnology*, *23*(5), 1459–1468.

Sheehan, J., Dunahay, T., Benemann, J., & Roessler, P. (1998). *A look back at the U. S. Department of Energy's aquatic species program: biodiesel from algae*. *National Renewable Energy Laboratory*.

Singh, J., & Gu, S. (2010). Commercialization potential of microalgae for biofuels production. *Renewable and Sustainable Energy Reviews*, *14*(9), 2596–2610.

Singh, P. P., & Saldaña, M. D. a. (2011). Subcritical water extraction of phenolic compounds from potato peel. *Food Research International*, *44*(8), 2452–2458.

Singh, S. P., & Singh, D. (2010). Biodiesel production through the use of different sources and characterization of oils and their esters as the substitute of diesel: A review. *Renewable and Sustainable Energy Reviews*, *14*(1), 200–216.

Skjånes, K., Rebours, C., & Lindblad, P. (2013). Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. *Critical Reviews in Biotechnology*, *33*(2), 172–215.

Smith, A. M., & Ross, A. B. (2016). Production of bio-coal, bio-methane and fertilizer from seaweed via hydrothermal carbonisation. *Algal Research*, *16*, 1–11.

Smith, R. M. (2002). Extractions with superheated water. *Journal of Chromatography A*, *975*(1), 31–46.

Soto Ayala, R., & Luque de Castro, M. . (2001). Continuous subcritical water extraction as a useful tool for isolation of edible essential oils. *Food Chemistry*, *75*(1), 109–113.

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, *101*(2), 87–96.

Subramanian, R., Nakajima, M., Kimura, T., & Maekawa, T. (1999). Membrane process for premium quality expeller- pressed vegetable oils. *Food Research International*, *31*(8), 587–593.

Suganya, T., & Renganathan, S. (2012). Optimization and kinetic studies on algal oil extraction from marine macroalgae *Ulva lactuca*. *Bioresource Technology*, *107*, 319–326.

Sunphorka, S., Chavasiri, W., Oshima, Y., & Ngamprasertsith, S. (2012). Kinetic studies on rice bran protein hydrolysis in subcritical water. *Journal of Supercritical Fluids*, *65*, 54–60. d

Thiruvenkadam, S., Izhar, S., Yoshida, H., Danquah, M. K., & Harun, R. (2015). Process application of Subcritical Water Extraction (SWE) for algal bio-products and biofuels production. *Applied Energy*, *154*, 815–828.

Toor, S. S., Reddy, H., Deng, S., Hoffmann, J., Spangsmark, D., Madsen, L. B., & Rosendahl, L. A. (2013). Hydrothermal liquefaction of *Spirulina* and *Nannochloropsis salina* under subcritical and supercritical water conditions. *Bioresource Technology*, *131*, 413–419.

Uddin, M. S., Ahn, H. M., Kishimura, H., & Chun, B. S. (2010). Production of valued materials from squid viscera by subcritical water hydrolysis. *Journal of Environmental Biology*, *31*(5), 675–679.

Vanthoor-Koopmans, M., Cordoba-Matson, M. V., Arredondo-Vega, B. O., Lozano-Ramírez, C., Garcia-Trejo, J. F., & Rodriguez-Palacio, M. C. (2014). Microalgae and Cyanobacteria Production for Feed and Food Supplements. In *Biosystems Engineering: Biofactories for Food Production in the Century XXI* (pp. 253–275).

Vijayabaskar, P., Vaseela, N., & Thirumaran, G. (2012). Potential antibacterial and antioxidant properties of a sulfated polysaccharide from the brown marine algae *Sargassum swartzii*. *Chinese Journal of Natural Medicines*, *10*(6), 421–428.

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 and Emerging Technologies*, *9*(2), 161–169.

Viriya-Empikul, N., Wiboonsirikul, J., Kobayashi, T., & Adachi, S. (2012). Effects of temperature and flow rate on subcritical-water extraction from defatted rice bran. *Food Science Technology Resource*, *18*(3), 333–340.

Wahyudiono, Machmudah, S., & Goto, M. (2013). Utilization of sub and supercritical water reactions in resource recovery of biomass wastes. *Engineering Journal*, *17*(1), 1–12.

Wijesekara, I., Pangestuti, R., & Kim, S. K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydrate Polymers*, *84*(1), 14–21.

Wolfe, R. R. (2002). Regulation of Muscle Protein by Amino Acids. *J Nutr*, *132*, 3219–3224.

Wu, H., & Wu, D. Y. (1925). Nature of Heat Denaturation of Proteins. *Journal of Biological Chemistry*, *64*(3), 369–378.

Yoshida, H., Terashima, M., & Takahashi, Y. (1999). Production of organic acids and amino acids from fish meat by sub-critical water hydrolysis. *Biotechnology Progress*, *15*(6), 1090–1094.

Zakaria, S. M., & Kamal, S. M. M. (2015). Subcritical Water Extraction of Bioactive Compounds from Plants and Algae: Applications in Pharmaceutical and Food Ingredients. *Food Engineering Reviews*, *8*(1), 23–34.

Zhao, G., Chen, X., Wang, L., Zhou, S., Feng, H., Chen, W. N., & Lau, R. (2013). Ultrasound assisted extraction of carbohydrates from microalgae as feedstock for yeast fermentation. *Bioresource Technology*, *128*, 337–344.

Zhuang, Q., Qin, Z., & Chen, M. (2013). Biofuel, land and water: maize, switchgrass or Miscanthus ? *Environmental Research Letters*, *8*(1), 1–6.