OPTIMIZATION OF ROSELLE SEED IN SUPERCRITICAL CARBON DIOXIDE

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All the glory to the Lord, and especially thanks to my beloved dad, mum, bi, supervisor and friends.

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ABSTRACT

Hibiscus sabdariffa (Roselle) seeds are high in proteins, unsaturated fatty acids, good source of minerals and antioxidants especially rich in gamma tocopherol. It reserves nutritional, industrial and pharmaceutical importance. To date, studies on the Roselle seed extraction are using conventional solvent extraction which usually take very long and is not favorable to extract active compound from heat sensitive samples. In this study, supercritical carbon dioxide (SC-CO_2) extraction was applied for extraction of oil from Roselle seeds at temperature range of 40°C to 80°C and pressure of 20 MPa to 30 MPa. The effects of temperature and pressure on the extraction yield, solubility of oil and gamma tocopherol content were determined. The optimization of Roselle seed oil extraction process was conducted using the response surface methodology in order to obtain the highest yield and gamma tocopherol content. Roselle seed with low moisture content of 8.03% were used to prevent the moisture from acting as mass transfer barrier. Particle size of 300 μm and SC-CO_2 flow rate at 5 ml/min was chosen for extraction and performed at 180 minutes constantly throughout this study. The overall oil yield increased with increased in pressure and temperature. While a reverse effect was observed with continuous rising of temperature. The highest Roselle seed oil recovery of 16.17% was obtained at 30 MPa and 40°C. The highest gamma tocopherol concentration of 5.6 mg per 100 g of extracted Roselle seed oil was observed at a low temperature of 40°C. The optimum extraction condition for Roselle seed oil was observed at pressure of 30MPa and temperature of 40°C. According to the analysis of variance, the coefficient determination R^2 for Roselle seed extraction oil yield and gamma tocopherol were 0.9723 and 0.9754 of the total variance is explained by the second-order polynomial model, indicating a good correlation and agreement between the experimental and predicted values. In the experimental range, the results showed that the Roselle seed oil yield, gamma tocopherol content and solubility are significantly influenced by temperature and pressure.

ABSTRAK

Biji *Hibiscus sabdariffa* (Roselle) didapati tinggi dengan protein, asid lemak tak tepu, sumber mineral yang baik dan antioksidan terutamanya kaya dengan tocoferol gamma. Ia mempunyai kepentingan dari sudut pemakanan, perindustrian dan farmaseutikal. Sehingga kini, kajian mengenai pengekstrakan biji Roselle hanya menggunakan pengekstrakan pelarut konvensional yang biasanya mengambil masa yang lama dan tidak menguntungkan untuk mengeluarkan sebatian aktif dari sampel sensitif haba. Dalam kajian ini, pengekstrakan karbon dioksida lampau genting (SC-CO_2) digunakan untuk pengekstrakan minyak dari biji Roselle pada julat suhu 40°C hingga 80°C dan tekanan 20 MPa hingga 30 MPa. Kesan suhu dan tekanan ke atas hasil pengekstrakan, kelarutan minyak dan kandungan tokoferol gamma ditentukan. Pengoptimuman proses pengekstrakan minyak biji Roselle dikendalikan menggunakan kaedah sambutan permukaan untuk memperoleh hasil tertinggi dan kandungan tokoferol gamma. Biji Roselle dengan kandungan kelembapan yang rendah iaitu 8.03% digunakan untuk mencegah kelembapan daripada bertindak sebagai penghalang pindah jisim. Saiz zarah 300 μm dan kadar aliran SC-CO_2 pada 5 ml / min dipilih untuk pengekstrakan dan dilakukan pada 180 minit ditetapkan sepanjang kajian ini. Hasil minyak keseluruhan meningkat apabila tekanan dan suhu meningkat. Manakala kesan sebaliknya diperhatikan pada peningkatan suhu yang berterusan. Perolehan minyak biji Roselle tertinggi sebanyak 16.17% diperoleh pada 30 MPa dan 40°C. Kepekatan tokoferol gamma tertinggi 5.6 mg per 100g minyak biji Roselle yang diekstrak diperhatikan pada suhu rendah 40°C. Keadaan pengekstrakan optimum untuk minyak biji Roselle diperhatikan pada tekanan 30 MPa dan suhu 40°C. Menurut analisis varians, penentuan pekali R^2 untuk hasil minyak ekstrak biji Roselle dan tokoferol gamma adalah 0.9723 dan 0.9754 dari jumlah varians dijelaskan oleh model polinomial tertib kedua, menunjukkan korelasi dan persetujuan yang baik antara nilai eksperimen dan nilai jangkaan. Dalam julat eksperimen, keputusan menunjukkan bahawa hasil minyak biji Roselle, kandungan tokoferol gamma dan kelarutan sangat dipengaruhi oleh suhu dan tekanan.

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LIST OF ABBREVATION

ALA	-	Alpha linolenic acid
ABTS	-	2,2-Azino-bis
BHT	-	Butylated hydroxytoluene
CCC	-	Canadian Canola Council
DOA	-	Department of Agriculture
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
EFA	-	Essential fatty acids
FAO	-	Food and Agriculture Organization of the United Nations
HPLC	-	High Performance Liquid Chromatography
LA	-	Linoleic acid
Meq	-	Milliequivalent
MPa	-	Megapascal
MPOB	-	Malaysia Palm Oil Board
MUFA	-	Mono-unsaturated fatty acid
NSAID		Non-steroidal anti-inflammatory drug
PUFA	-	Poly-unsaturated fatty acid
RSM	-	Response surface methodology
SC-CO_2	-	Supercritical carbon dioxide
SFE	-	Supercritical fluid extraction
TTP	-	Tocopherol transfer protein

UN	-	United Nations
USDA	-	United States Department of Agriculture
UV	-	Ultraviolet

LIST OF SYMBOLS

α	-	Alpha
β	-	Beta
δ	-	Delta
γ	-	Gamma
k	-	number of factor
N	-	number of experiment
P_c	-	Critical Pressure
T_c	-	Critical temperature
Y	-	Extracted Oil Yield
Y_{calc}	-	Solubility data calculated using density based models
Y_{exp}	-	Solubility data obtained from experiment
W_f	-	weight of sample bottle and extracted oil
W_i	-	weight of empty sample bottle
W_{oil}	-	weight of extracted oil
W_s	-	weight of sample used
°C	-	Degree Celcius
%	-	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Oil seed corps is the important sources of natural oil for human nutrition, pharmaceutical and industries. The global demand of seed oil is increasing every year due to the growing world population and their usage for industrial purpose (USDA, 2017). Each seed oils have different characteristic to determine their purposes which depend on the composition of the fatty acid. Seed oils such as palm oil, coconut oil, soybean oil, castor oil, rapeseed oil and sunflower seed oil are popular used for industrial purposes in manufacturing of soaps, detergents, lubricants, solvents, paints, inks, surfactants and cosmetics (Damude *et al*., 2008; MPOB, 2017; Patel *et al*., 2016).

Seed oils are rich in proteins, fatty acids, minerals, fibers and vitamins that are important for human health. Presently, scientists are looking for new seed oil to meet the growing demand for healthy oils especially seed oil with fatty acids of oleic (Omega-9), linoleic (Omega-6) and linolenic (Omega-3) acids. These are the essential fatty acid (EFA) which human bodies could not synthesize and can be obtained from external sources in diet. The EFA is important for producing life energy (Burr *et al*., 1932; Bill, 2012), studies showed that many common illness like hypercholesterolemia, low immune system, low metabolism rate are related to imbalances or deficiencies of these EFA (Sarwar *et al.,* 2013; Bradberry *et al*., 2013).

In addition, the Omegas are the building blocks of healthy cell membranes. The polyunsaturated fatty acids (PUFA) are skin's natural oil barrier and critical components to keep skin hydrated (Lin *et al*., 2018). Seed oils are widely used in cosmetic and skin care products, as the Omega-3 and Omega-6 fatty acids are reported to keep skin moisturized, reduce the transepidermal water loss, aid in healing process of acne and sunburns (Zielinska *et al.*, 2014; McCusker *et al.*, 2010). Oleic acid is reported to be effective percutaneous absorption enhancer which increase the penetration of non-steroidal anti-inflammatory drug (NSAID) by interaction with subcutaneous lipids (Kim *et al.*, 2008).

The additonal benefits from seed oil is the richness of tocopherols content. Vitamin E is an important dietary nutrient for humans due to it is only synthesized by plant and most of the sources are from seed oil extraction. Tocopherols include alpha, beta, delta and gamma isomers. They are all chain breaking antioxidants with scavenging activities towards the free radicals of unsaturated lipids (Yamauchi, 1997). Alpha-tocopherol is the most bioactive isomer which reported as the first defence to prevent lipids from peroxidation in living cell membranes function. Gamma tocopherol is another isomer found abundantly in seed oil which present more superior antioxidant level compared to alpha-tocopherols. Reports have shown it protects cardiovascular system and inversely association of plasma gamma-tocopherol concentration with coronary disease (Cooney *et al.,* 2008; Marchese *et al.*, 2014).

As the awareness of using healthy oils in both diet and personal care products increases, the demand of seed oils in natural products industries increases drastically. New finding for seed oils have been done in countries. according to Abdul Afiq *et al,* (2013), in Arab the date seed oil was extracted for dietary seed oil alternative. In South Africa, Baobab (*Adansonia digitata* L.) seed oil was in high interest to be used in cosmetic industry (Vermaak *et al.*, 2011; Komane *et al.*, 2017). Scientist in West Africa were exploring the potential uses of oil extracted from *L. kerstingii* seeds which is usually used as traditional medicine to promote the consumption in local communities (Judicael *et al.*, 2017).

There is a native plant in Malaysia with high potential of its seed oils extraction for healthier oil alternative. Hibiscus *sabdariffa* L. (Roselle), which is one of the most popular plant grown worldwide in India, Mexico, Sudan, Saudi Arabia, Indonesia, Thailand, Philippines, Vietnam, Africa, Taiwan and Egypt. The plant take only three to four months to reach the commercial stage of maturity where the calyces are harvested.

Roselle is one of the commercial crops that are mostly exploited for food processing industry in Malaysia. However, the increasing of health awareness on reduce processed food have reduced the demand of Roselle. Currently, the utilization of Roselle plant focused on the calyces only. Calyces are removed by peeling off the petals of the mature flowers from the capsule containing the Roselle seeds. After removing the petals, the other parts are throw as waste or used as plant food by mixing in the soil for further plantation. Explore the other novel part of Roselle plant is one of the ways helping the farmer to harvest all the matured Roselle and to reduce the plantation waste. The red calyces of Roselle have also drew all the attention of the academician and pharmaceutical applications in past decades. The extract is in attractive red color and the tastety flavour make Roselle calyces a valuable products in food processing for the preparation of jam, jellies and beverages. Besides, it is used as a traditional medicine to treat hypertension, cancer, cough, fever and scurvy (Ologundudu *et al*., 2010; Muhammad *et al*., 2008). The antioxidant can be good component in cosmetic and so forth. These make Roselle calyces become a popular research by scientists. There are many reported information on its characteristics, nutritional properties, vitamins content, antioxidant activities and the optimum extraction process can be easily found in literature.

Beside the calyces, studies regarding the other parts of Roselle remained unpopular. Roselle seed which is usually discarded after mature calyces harvested was previously reported high in nutritional value, especially oleic-linoleic group of fatty acids and the tocopherols content. Some studies had significant shown that Roselle seed oil has higher protein, dietary fiber, and minerals compared to other seeds like passion fruit and black seeds (Ismail *et al*., 2008). The tocopherol in Roselle seed oil is 4 times the concentration in saffflower and 20 fold higher than that in grapeseed oil, with up to 25% of alpha tocopherols, 5% delta tocopherols and 70% of gamma-tocopherols in total tocopherol content (Mohamed *et al*., 2007).

These previous investigations established the potential of Roselle seed oil utilization in food, pharmaceutical and cosmetic industries.

There are lack of studies regarding the optimum extraction process of Roselle seed oil to produce highest yield and quality oil which causes the limitation of seed oil use in industries. Although the conventional methods are popular enough for seed oil extraction, but there are reports showing the disadvantages such as the solvent residual in extract, long extraction time and low active compounds extracted (Yan, 2013; Han *et al*, 2013; Jibrin *et al*., 2014). These limitation lead to significant increase in the demand for appropriate, selective, cost saving and eco-friendly extraction techniques that can perform faster, produce higher yield and better quality extract. Few years ago, supercritical fluid extraction (SFE) have attracted the attention as an alternative to conventional technique. It has been widely used to extract various natural compounds. Supercritical fliud technology is recognized as an effective technique that offer these stated benefits. The extraction solvent in supercritical fluid extraction is a fluid at a temperatue and pressure above the critical value. It has been used for laboratory-scale level to preparative scale, pilot scale and up to larger scale industrial commercial production.Qualitative and quantitative data such as constituents of natural products, concentration of active compounds can be obtained by little adjustment to factors like extraction temperature, pressure, solvent flow rate, cosolvemt addtion and sample volume. Hence in this study, supercritical carbon dioxide (SC-CO_2) on Roselle seed oil extraction is studied and optimized to obtain high purity oil with high gamma tocopherol content by obtaining more information on the extraction processes and mechanisms.

1.2 Problem Statement

Studies showed Roselle seed have the potential nutritional benefits in food, pharmaceutical and cosmetic industries. The oil extracted from Roselle is an attractive natural oil for cooking, dietary supplement or skin health purposes. Unfortunately, there are lack of clear understanding on the extraction process for scaling-up the production in order to produce a high quality tocopherol enriched Roselle seed oil.

Most of the reported Roselle seed oil extraction were using the conventional solvent extraction which is the most common method that usually benefits by more sample mass extracted than using other methods. However, there are reports showing the disadvantages of potential toxic solvents contaminate the sample extract during the process and there are large amount of solvent wasted. Besides, the extraction process is very long, usually take 6-12 hours to complete a cycle of extraction which is not economically friendly. Conventional solvent extraction usually conduct under high temperature which is not suitable for heat-sensitive sample. Studies showed the active compounds extracted using conventional method is much lesser compared to other extraction technology. Especially the gamma tocopherol contain in Roselle seed oil is sensitive to the extraction temperature. Hence, a high selective extraction method must be used in order to obtain high quality extract.

In this study, supercritical carbon dioxide (SC-CO_2) extraction is proposed for Roselle seed oil extraction. The SC-CO_2 produce no hazardous waste to environment since the solvent use is carbon dioxide, the gas is inexpensive and can be recycle for next use. The special fractionation capabilities allow specific compound to be extracted by operating well the extraction parameters.

There lack of research conducted to understand the Roselle seed oil extraction process using SC-CO_2 extraction. Therefore in this study, the tocopherol enriched Roselle seed oil will be extracted and the optimization of the extraction parameters, such as the particle size, extraction time, solvent flow rates, temperature, and pressure will be carried out using response surface methodology (RSM) in order to obtain the optimum conditions to extract the highest yield and tocopherol rich Roselle seed oil.

1.3 Objectives

The objectives of this research are:

i. To determine the effect of temperature and pressure on the Hibiscus sabdariffa L. (Roselle) seed oil yield, solubility and gamma tocopherol concentration using supercritical carbon dioxide ($SC\text{-}CO_2$) extraction.

ii. To optimize the supercritical extraction conditions on the Hibiscus sabdariffa L. (Roselle) seed oil yield, solubility and gamma tocopherol concentration using response surface methodology (RSM).

1.4 Scope of Study

The scopes of this research are:

1. The Roselle seed oil is extracted using supercritical carbon dioxide ($SC\text{-}CO_2$) and Soxhlet extraction.
2. The pretreatment process for $SC\text{-}CO_2$ extraction involve sample preparation, determination of optimum particle size of seed, extraction time and $SC\text{-}CO_2$ flow rate were performed prior to extraction in order to obtain the constant parameters.
3. The effect of extraction temperature and pressure on extraction oil yield, solubility and gamma-tocopherol concentration in Roselle seed oil were studied.
4. The concentration of gamma-tocopherol in Roselle seed oil obtained from $SC\text{-}CO_2$ and Soxhlet extraction was analyzed quantitatively using the method of high-performance liquid chromatography (HPLC).
5. The extraction oil yield and concentration of gamma tocpherol in Roselle seed oil extracted using $SC\text{-}CO_2$ was studied and compared to seed oil extracted using Soxhlet extraction method.
6. The optimum conditions for the extraction of gamma tocopherol rich Roselle seed oil using $SC\text{-}CO_2$ extraction were determined. The optimization process was studied using response surface methodology (RSM).

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