

OPTIMIZATION OF ARECA CATECHU SEED OIL EXTRACTED BY
SUPERCRITICAL CARBON DIOXIDE (SCCO₂) USING PRINCIPLE
COMPONENT ANALYSIS (PCA)

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To my beloved family and friends

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ABSTRACT

A study on the optimization of supercritical carbon dioxide (SCCO₂) on *areca catechu* seed oil was carried out at temperature and pressure ranging from 50 °C to 80 °C and 20 MPa to 30 MPa respectively. Three grams of ground *areca catechu* seeds with particle size of 177.5 µm and moisture content of 10.95% were used in the SCCO₂ extraction process that was carried out for 60 minutes. Fourier transform infra-red spectroscopy (FTIR) analysis was applied to determine the profile of SCCO₂ extracts in each condition. A chemometrics method of Principal Component Analysis (PCA) was applied to the FTIR spectra to optimize the condition for catechin extraction. The scores and plot show that, the highest quality of catechin was detected in the sample extracted at 70 °C and 30 MPa. Whereas the lowest quality of the catechin was detected in the sample extracted at 50 °C and 20 MPa. The highest concentration of catechin was found in the sample extracted at 50 °C and 20 MPa. Overall, the sample extracted at 70 °C and 30 MPa possessed the highest quality of catechin in low concentration. The *areca catechu* oil was further analyzed for the physicochemical properties. The Acid value of *areca catechu* is 18.7 ± 0.06 mg NaOH / g oil or 9.397 % free fatty acid as oleic acid and iodine value is 69.54 ± 0.57 g I₂/100g. Whereas peroxide and saponification values are 6.5 mequiv.O₂/kg and 173.91 ± 0.64 mg KOH/g respectively. A thermogravimetric analysis was used to study the pyrolysis temperature range. The decomposition of the sample started at 160 °C and ended at 470 °C. Whereas the Gas Chromatography Mass Spectrometer (GCMS) analysis detected 12 fatty acids in *areca catechu* oil sample. There are 8 saturated fatty acids consisting of caprylic acid (C_{8:0}), capric acid (C_{10:0}), lauric acid (C_{12:0}), myristic acid (C_{14:0}), palmitic acid (C_{16:0}), stearic acid (C_{18:0}), arachidic acid (C_{20:0}) and behenic acid (C_{22:0}) and 4 unsaturated fatty acid consisting of palmitoleic acid (C_{16:1}), oleic acid (C_{18:1}), linoleic acid (C_{18:2}) and α -linolenic acid (C_{18:3}).

ABSTRAK

Kajian pengoptimuman superkritikal karbon dioksida (SCCO₂) pada minyak benih pinang (*Areca Catechu*) telah dijalankan pada suhu dan tekanan dari 50 hingga 80 °C dan 20 hingga 30 MPa. Tiga gram serbuk benih *areca catechu* yang bersaiz 177.5 µm dengan kandungan kelembapan sebanyak 10.95% telah digunakan dalam proses pengekstrakan SCCO₂ yang telah dijalankan selama 60 minit. Fourier transform infra-red spectroscopi (FTIR) analisis telah digunakan untuk menganalisis profil ekstrak SCCO₂. Salah satu daripada kaedah Analisis Komponen Utama (PCA) iaitu kemometrik digunakan pada spektrum FTIR untuk menganalisis keadaan optimum pengekstrakan katekin. Skor dan plot yang didapati menunjukkan bahawa katekin yang berkualiti tinggi dikesan dalam sampel yang diekstrak pada 70 °C dan 30 MPa dan diikuti oleh sampel diekstrak pada 80 °C dan 30 MPa. Manakala katekin yang berkualiti rendah dikesan dalam sampel pada 50 °C dan 20 MPa dan kepekatan tertinggi katekin terdapat dalam sampel pada 50 °C dan 20 MPa. Secara keseluruhan, sampel diekstrak pada 70 °C dan 30 MPa mempunyai katekin yang berkualiti tinggi dalam kepekatan yang rendah. Minyak *areca catechu* dianalisis untuk sifat-sifat fizikokimia. Nilai asid *areca catechu* adalah 18.7 ± 0.06 mg NaOH/g atau 9.397% asid lemak dan nilai iodin sebagai nilai oleik adalah 69.54 ± 0.57 g I₂/100g. Manakala, nilai peroksida dan nilai penyabunan adalah 6.5 mequiv.O₂/kg dan 173.91 ± 0.64 mg KOH/g. Penguraian sampel bermula pada 160 °C dan berakhir pada 470 °C. Manakala, analisis Gas kromatografi mass spectrometry (GCMS) mengesan 12 asid lemak dalam sampel minyak *areca catechu*. Terdapat 8 asid lemak tepu yang terdiri daripada asid kaprilik (C_{8:0}), asid kaprik (C_{10:0}), asid laurik (C_{12:0}), asid myristik (C_{14:0}), asid palmitik (C_{16:0}), asid stearik (C_{18:0}), asid arakidit (C_{20:0}) dan asid behenik (C_{22:0}) dan 4 asid lemak tak tepu terdiri daripada asid palmitoleik (C_{16:1}), asid oleik (C_{18:1}), asid linoleik (C_{18:2}) dan asid α-linolenik (C_{18:3}).

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LIST OF ABBREVIATIONS

SCCO ₂	-	Supercritical Carbon Dioxide
SF or SCF	-	Supercritical Fluid
NMR	-	Nuclear Magnetic Resonance
FTIR	-	Fourier Transform Infrared
GCMS	-	Gas Chromatography Mass Spectrometry
FAME	-	Fatty Acid Methyl Ester
PCA	-	Principle Component Analysis
PLS	-	Partial Least Square
CP	-	Critical Point
TP	-	Triple Point
T _c	-	Critical Temperature
P _c	-	Critical Pressure
DTA	-	Differential Thermal Analysis

LIST OF SYMBOLS

T_c	-	Critical Temperature ($^{\circ}\text{C}$ or K)
P_c	-	Critical Pressure ($^{\circ}\text{C}$ or K)
ρ	-	Density (kg/m^3)
ρ_c	-	Critical Density (kg/m^3)
F	-	Flow Rate (mL/min)
t_e	-	Extraction Time (sec or min)
d_p	-	Particle Size (μm)

CHAPTER 1

INTRODUCTION

1.1 Introduction

Studying on the natural products especially on plants is always an interesting objective for scientist. Throughout history, it is proved that, plants have been a good source in providing various useful compounds such as vitamin, phenolic and nitrogen along with metabolites that are rich in bioactivities such as antioxidant, anti-carcinogenic, anti-bacterial and anti-inflammatory. Due to this, plants have been the targeted group for researchers in identifying and developing the natural colorants, biodiesel, new drugs and much more with no or less side effect. This perspective increases the demand and requirement for plant studies by natural product researcher.

The plant that was selected for the study is *Areca Catechu* seed. *Areca Catechu*, which is commonly known as betel nut, is one of the most popular plant in tropical countries and it is commonly chewed within ethnic and cultural subgroups. Previous studies by Xing *et al.* (2010) showed that the area nut contains 50 to 60 % of sugars, 15 % of lipids (glyceride of lauric, myristic and oleic acid), 15 % condensed tannins (phlobatannin, catechin), polyphenolics and 0.2 to 0.3 % alkaloids (arecoline, arecaidine, guvacine and guvacoline). *Areca Catechu* is an important economical seed crop and it is widely cultivating in tropical and subtropical areas.

Areca Catechu is also known as a folklore medicine for various illnesses such as dyspepsia (indigestion), constipation, beriberi and oedema. There are findings that the *Areca Catechu* possesses properties such as psychoactive, antidepressant, anthelmintic, anti-inflammatory, and antioxidant and so on (Xing *et al.*, 2010). Han *et al.* (2012) stated that *Areca Catechu* is rich in properties such as antibacterial, antifungal, antiviral, anti-aging, lower cholesterol and antioxidant activity. However, large amount of *Areca Catechu* can lead to various cholinergic effects such as salivation, lacrimation, urinary incontinence, sweating, and diarrhea or even can lead to cardiac arrhythmia (Li *et al.*, 2010).

Researcher based in India studied the properties of *Areca Catechu* seed and concluded that it possess characteristic such as collective inducible nitric oxide synthase (iNOS) inhibitory, analgesic, anti-inflammatory, anti-oxidant and anti-migraine activity (Bhandare *et al.*, 2010). Antioxidant activity of *Areca Catechu* is also mainly focus by researcher in all over the world (Xing *et al.*, 2010; Li *et al.*, 2010; Han *et al.*, 2012). Meanwhile, Dar *et al.* (1997) improved the antidepressant activity of *Areca Catechu*. The investigation on anti-aging effect of *Areca Catechu* resulted in improving the skin hydration, wrinkle reduction and skin elasticity (Phaechamud and Chitrattha., 2012). Therefore, it is clearly shows that *Areca Catechu* is gaining attention of researcher from various fields but there is no official documentary about the details of physicochemical properties, thermal stability and GCMS studies of *Areca Catechu* seed.

Besides, *Areca Catechu* is one of the plant that rich in Catechin. Catechin, which is a monomeric flavonoid was reported to have hydroxyl, peroxy, superoxide and DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activities (Gogoi *et al.*, 2010). Flavonoids are found in roots, leaves, stalks and fruits. Around 2 % of the materials formed by photosynthesis in the plant body are transformed to flavonoids, which function as a protector to defend from the ultraviolet lights and bacteria.

Flavonone, flavone, flavonol, catechin and epicatechin are the product of flavonoids, which is called “chalcone” (Cheong *et al.*, 2005). There are number of studies regarding the extraction of catechin using Supercritical carbon dioxide (SCCO₂) and soxhlet extraction. Li *et al.* (2010) studied the supercritical fluid extraction of catechin from *Cratoxylum prunifolium*. Meanwhile Bimakr *et al.* (2008) study the SCCO₂ extraction of Catechin, Epicatechin, Rutin and Luteolin from Spearmint (*Mentha spicata L.*) leaves. Whereas Chang *et al.* (2000) studied the separation of catechin from green tea using carbon dioxide extraction and concluded that Soxhlet ethanol extraction yield the largest amount of polyphenol. Cheong *et al.* (2005) studied the determination of catechin compounds in Korean green tea infusions under various extraction conditions by high performance liquid chromatography and concluded that the soxhlet extraction gave lower flavonoid concentration.

Based on the previous researches, it is proved that the beneficial effects of catechin, especially due to their biological activities received a great attention. The very specific and notable importance of catechin is its ability to restore and repair vitamin E. Catechin from green tea prevent some skin, liver, lung, gastric and breast cancer. It is also proved to reduce in cardiovascular disease, dental decay, obesity and development in immune system. This is due to its potential to prevent lipid peroxidation of oil and scavenge free radicals to stop the auto oxidative degradation of lipids. Likewise, the antioxidant property of catechin is also utilized to scavenge free radicals from detrimental biomolecules, chelate catalytic metal ions from free radical establishment. These functions preclude DNA damages by reactive oxygen species (Omar *et al.*, 2010).

Meanwhile, uses of catechin as a natural element in foodstuffs and feedstuffs for versatile function is also increasing dramatically particularly in Asia. The antioxidant properties help to get certain component to hold back in a food matrix (Bhandare *et al.*, 2010). The catechin from green tea in food components increase the

shelf life of the product and at the same time contributes to health benefits (Chang *et al.*, 2000).

In pharmaceutical industry, catechin is used to enhance oral health by adding to toothpastes, mouthwashes and breath fresheners. Presently, cosmetic products such as shampoos, moisturizing creams, perfumes and sunscreens are added with catechin for protection from free radical damage and soothing effects on the skin (Chang *et al.*, 2000). Normally, researchers will mainly focus on matters such as extraction comparison and optimization of particular compounds. But, to the best of our knowledge, there is a missing part in the effect of SCCO_2 conditions such as temperature and pressure on catechin extracted from *Areca Catechu* seed.

The conventional separation methods such as water extraction, solid phase extraction and steam distillation are commonly used for extraction of natural compounds. These techniques require long extraction time, labour intensive operation, low yield, toxic solvent residue and degradation of thermo sensitive compounds (Bimakr *et al.*, 2008). Besides, most of these extraction processes are executed by a plethora of organic solvents as extractants or partitioning phases. Usage of organic solvents increases the storage and disposal costs associated with the used solvent, exposure of laboratory personnel to harmful solvents, problems with collection and storage of waste solvents. Most importantly, organic solvents are very destructive to the environment. The public awareness of health, environment and safety contributes to the issue of minimization in the usage of organic solvents to a critical focus. Therefore, it is very important to satisfy the safety standards and regulatory requirements. The disadvantages of conventional methods were overcome by the advanced technologies such as supercritical fluid extraction, membrane separation, ultrasonic assisted extraction, accelerated solvent extraction, molecular distillation and much more. This study will focus on supercritical fluid extraction with carbon dioxide as solvent.

Supercritical fluid extraction was employed as an excellent alternative for isolation of the worthwhile organic from plants. The specific property of supercritical fluid is high in isothermal compressibility compare to liquids. Good solvability of supercritical fluid cause high density for the fluid. In addition, changes in pressure and temperature change the solubility in a broad range. Hence, the extracted substance can be separated from solvent just by apply changes in pressure or temperature under critical point. Besides, supercritical fluid with low critical temperature can extract substances that are sensible to temperature without any destruction (Zarinabadi *et al.*, 2010).

Application of carbon dioxide as supercritical fluid is rapidly growing due to its advantages over other supercritical fluids. Carbon dioxide is a nonflammable and it is naturally abundant with a TLV (Threshold limit value) of 5000 ppm for airborne concentration at 298K (Beckman., 2004). Carbon dioxide is also chemically inert, low toxicity, no pollution, shorter concentration time, non-corrosive, environmentally friendly, cheap and readily available in bulk quantity with high purity. Low critical temperature of carbon dioxide, which is 31°C in the process, is near to the environmental temperature and it is easily removed from the extracted products. Hence, it is minimizing the needs for the heat demand and decrease the thermal damage to the tested compounds. Due to its incompatible features, SCCO₂, had become the most acceptable supercritical solvent in various industries. Therefore, the uses of SCCO₂ reduce the extraction costs and CO₂ is recycled and reuse in the process.

1.2 Problem Statement

Catechin that present in *Areca Catechu* seed oil is rich in various medicinal properties and it is potential to apply in other fields. Previous researcher proved that, the catechin present as a major component in *Areca Catechu* seed through various extraction methods. However, until to date, there is no studies have been done to determine the optimum condition of SCCO₂ extraction that gives the highest yield of the quality catechin. The main goal in this research is to determine the optimum condition of SCCO₂ extraction that gives the highest yield of quality catechin and the physicochemical properties.

1.3 Objective

- To investigate the effects of pressure and temperature of SCCO₂ extraction on oil yield extracted from *Areca Catechu* seeds.
- To optimize the supercritical carbon dioxide (SCCO₂) extraction condition on *Areca Catechu* seed by Chemometrics using FTIR data.
- To determine the fatty acid methyl ester (FAME) and investigate the physicochemical properties of *Areca Catechu* seed oil extracted by SCCO₂.

1.4 Scope of Research

In order to achieve the objective of this study, the following scopes were followed:

- I. Sample preparation
 - The *Areca Catechu* sample were powdered before proceed with the extraction process. The particle size of the *Areca Catechu* seeds are varies from 177.5 – 755 μm .
- II. SCCO₂ Extraction
 - The SCCO₂ parameter used in the extraction process are temperature (50 – 80 °C), pressure (20 – 30 MPa), CO₂ flow rate (2 - 4 ml/min) with constant extraction time of 60 minutes.
- III. Determination of functional group and optimum condition for the extraction
 - The Fourier Transform Infrared Spectroscopy is used to identify the functional group and fingerprint.
 - The discrimination and classification from Chemometrics by FTIR data will be used to identify the optimum condition for the extraction of catechin from *Areca Catechu* seeds.
- IV. Determination of physicochemical properties and thermal stability
 - The physicochemical properties are such as acid value, iodine value, saponification value, peroxide value and thermal stability are determined.
- V. Determination of Fatty acid methyl ester (FAME)
 - Gas chromatography mass spectrometry (GCMS) is used to identify the Fatty acid methyl ester (FAME) that present in *Areca Catechu* seed oil

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