



**EXTRACTION, OPTIMIZATION AND CHARACTERIZATION OF INCA
INCHI OIL AND ITS OIL MEAL FOR CHEWABLE OIL TABLETS AND
COOKIES FORMULATION**

By

CHRISTOPHER JEFFI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

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July 2024

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Inca Inchi Oil (*Plukenetia volubilis* L.), rich in essential fatty acid faces challenges that limit its practical applications. These include susceptibility to oxidation, low extraction yields and quality from conventional methods. This study aims to enhance oil yield, antioxidant properties, and quality through heat pretreatment (microwave and hot air oven) and enzyme-assisted extraction, develop chewable oil tablets, and evaluate the acceptability of its press cake in cookies. The study showed that microwave pretreatment of inca inchi seeds for 4 min resulted in the highest oil yield (43.39%), compared to control (37.76%). Pre-treated oil samples had better quality in terms of free fatty acids (<1%) and peroxide values (<8 meqO₂/kg). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) study, showed that pretreated oil had good radical scavenging activity (70% and 90%). The extraction efficiency of the enzymes Neutrase 0.8L and Viscozyme L were evaluated. The study showed that, among the enzymes, Neutrase 0.8L was more efficient in extracting the oil (46.77%) when compared to Viscozyme L (14.7%). The

oil extracted had low free fatty acid (<1%) and peroxide value (<8 meqO₂/kg). The oil showed high scavenging activity (73% and 82%) as analyzed by DPPH and ABTS assays. Spray drying was used to develop microcapsules of blends of inca inchi oil-virgin coconut oil and inca inchi oil-red palm oil. Evaluation of the microcapsules showed that the microcapsules exhibited good flowability and low moisture content (<3%). The microcapsules showed reduction in fatty acids and the retention of β carotene was about 60%. Through the process of optimization, high encapsulation efficiency (>90%) was achieved at 40% and 160°C for total solids and inlet air temperature, respectively for both the samples. Chewable oil tablets were developed from spray dried encapsulates by direct compression technique. Analyses of the tablets in terms of attributes such as hardness, disintegration time and friability showed that the tablets were within the standard value as recommended by United States Pharmacopoeia. The invitro release of oil showed that the % of oil release was higher in gastrointestinal condition (8.5-18.67%) than in gastric condition (6.2-7.78%). Storage studies on the attributes of the chewable tablets indicated significant differences ($p < 0.05$) in the attributes, but they remained within the acceptable limits over two months at 4°C and 25°C. Additionally inca inchi oil press cake at various proportions (5%, 10%, 20% and 30%) were used to develop cookies. The study observed that cookies containing 10% oil meal had high protein content (13.03%) against control (4.89%). The hardness of the cookies formulated with press cake were low (2.52-3.22 N) compared to control (3.30 N). Moisture stability of the cookies stored for a month showed that water activity remained below 0.6. Sensory analysis favored cookies with 10% formulation, suggesting its potential for developing healthier cookies. Overall, the study shows that by using innovative extraction techniques, developing dietary supplements, and incorporating press cake into food

products, the study paves the way for its application benefiting both consumers and the industry.

Keywords: Chewable oil tablets, cookies, enzymes; inca inchi oil; spray drying

SDG: GOAL 3: Good Health and Well-Being, GOAL 12: Responsible Consumption and Production



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENGEKSTRAKAN, PENGOPTIMUMAN DAN PENCIRIAN MINYAK
INCA INCHI DAN BUNGKILNYA DALAM PEMBENTUKAN TABLET
MINYAK KUNYAH DAN FORMULASI BISKUT**

Oleh

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Minyak Inca Inchi (*Plukenetia volubilis*. L), kaya dengan asid lemak penting, mempunyai aplikasi yang berpotensi dalam bidang terapeutik, makanan dan farmaseutikal. Walau bagaimanapun, kerintangannya terhadap pengoksidaan menghalang aplikasinya. Kajian ini meneroka kaedah pengekstrakan dan pengkapsulan melalui pengeringan semburan untuk membangunkan tablet minyak kunyah. Kajian juga melihat kebolehterimaa tepung inca inchi dalam biskut untuk mempelbagaikan penggunaannya dalam produk makanan. Pengekstrakan minyak menggunakan teknik konvensional mempunyai had dalam memaksimumkan hasil minyak. Kajian dijalankan untuk menentukan kesan prarawatan haba dalam bentuk ketuhar gelombang mikro dan udara panas ke atas hasil, kualiti minyak, antioksidan, dan komposisi asid lemak. Keputusan menunjukkan rawatan gelombang mikro selama 4 minit menghasilkan hasil minyak tertinggi (43.39%) berbanding kawalan (37.76%). Sampel minyak pra-rawatan menunjukkan asid lemak bebas dan nilai peroksida yang lebih rendah daripada kawalan. Kajian 2,2-diphenyl-1-picrylhydrazyl (DPPH) dan

2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonik acid (ABTS), menunjukkan minyak mempunyai aktiviti penghapusan radikal yang baik (70% dan 90%, masing-masing). Tambahan pula, pengekstrakan bebas pelarut menggunakan hidrolisis enzimatik dengan Neutrase 0.8 L dan Viscozyme L telah diterokai. Metodologi Permukaan Tindak Balas (RSM) digunakan untuk mengoptimumkan dos enzim (2-3.5%), masa (1-5 jam), dan suhu pengeraman (40-50°C). Kajian mendapati Neutrase 0.8 L lebih cekap daripada Viscozyme L dalam mengekstrak minyak. Kecekapan pengekstrakan tertinggi (47%) dicapai dalam masa optimum (5 jam), suhu pengeraman (40 °C), dan dos enzim (3.5%). Profil asid lemak dan kualiti minyak tidak terjejas dan berada dalam julat (ω -3:48.0-50.8%; ω -6:33.0-41%; ω 9: 6.8% -11%; Palmitik acid:4.0-4.9%; Stearic acid: 2.0-3.68%). Nilai peroksida dan asid lemak bebas adalah <10 meq O₂/kg; dan 5%, masing-masing. Pengeringan semburan meningkatkan kestabilan minyak dengan melindungi komponen teras. Kajian ini membangunkan mikrokapsul kering semburan campuran minyak inca inchi -minyak kelapa dara, dan minyak inca inchi -minyak sawit merah. Proses ini dioptimumkan dengan memilih jumlah pepejal (20-40%) dan suhu udara masuk (160-180°C) sebagai pembolehubah dan kecekapan pengkapsulan sebagai faktor tindak balas. Keputusan menunjukkan hasil serbuk sederhana (56.05-77.06%) dengan pengurangan ketara dalam asid lemak dan karotenoid. Kecekapan pengkapsulan tinggi (> 90%) dicapai pada 40% dan 160°C untuk jumlah pepejal dan suhu udara masuk untuk kedua-dua sampel. Tablet minyak kunyah diperkaya asid lemak penting dan karotenoid dihasilkan daripada pembungkusan kering semburan dengan teknik pemampatan terus. Pelepasan in vitro minyak menunjukkan % pelepasan minyak lebih tinggi dalam gastrousus (sehingga 18%) berbanding dalam gastrik (sehingga 10%). Kajian penyimpanan sifat-sifat tablet kunyah menunjukkan perbezaan ketara ($p < 0.05$) dalam tablet minyak kunyah, tetapi

ia kekal dalam had yang boleh diterima selama dua bulan pada 4°C dan 25°C. Akhir sekali, kandungan protein tinggi dalam tepung minyak membawa kepada penerokaannya dalam pembangunan biskut. Kajian menunjukkan biskut yang diformulasikan dengan tepung minyak menunjukkan kandungan protein yang tinggi berbanding kawalan (13.03% vs 4.89%) dan kekerasan berkurangan (2.52-3.22N) berbanding kawalan (3.30N). Analisis deria mengutamakan biskut berformulasi 10%, mencadangkan potensinya dalam pembangunan biskut yang lebih sihat. Kesimpulannya, kajian menunjukkan kepelbagaian minyak inca inchi dalam pelbagai aplikasi. Teknik pengekstrakan, pengkapsulan dan formulasi yang dibangunkan menunjukkan potensinya menangani keperluan pemakanan seperti pengurusan disfagia dan peningkatan profil pemakanan biskut. Penemuan ini membuka ruang untuk penggunaan minyak inca inchi dan produk sampingannya dalam makanan fungsian, memenuhi keperluan terapeutik dan pemakanan.

Kata Kunci: Biskut, enzim, minyak inca inci, pengeringan semburan, tablet minyak kunyah

SDG: MATLAMAT 3: Kesihatan yang Baik dan Kesejahteraan, MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawab

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))
AI	Active Ingredient
ALA	Alpha-Linolenic acid
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemist Society
CI	Carr's Index
DPA	Docosapentaenoic Acid
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
DSC	Differential Scanning Calorimetry
EMC	Equilibrium Moisture Content
EP	European Pharmacopeia
EPA	Eicosapentaenoic Acid
FCCD	Face Centered Central Composite Design
FFA	Free Fatty Acid
FID	Flame Ionization Detector
GA	Gum Arabic
GC	Gas Chromatography
HDL-C	High-Density Lipoprotein Cholesterol
HPLC	High Performance Liquid Chromatography
HR	Hausner Ratio
II	Inca Inchi
IIO	Inca Inchi Oil
IIOPC	Inca Inchi Oil Press Cake
IV	Iodine Value
LCT	Long-Chain Triglycerides

LDL-C	Low-Density Lipoprotein Cholesterol
MAE	Microwave Assisted Extraction
MD	Maltodextrin
MEE	Micro Encapsulation Efficiency
MUFAs	Mono Unsaturated Fatty Acids
OAC	Oil Absorption Capacity
PUFAs	Poly Unsaturated Fatty Acids
PV	Peroxide Value
PVA	Provitamin A
QE	Quercetin Equivalent
RPO	Red Palm Oil
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
SFAs	Saturated Fatty Acids
SGC	Simulated Gastric Condition
SGF	Simulated Gastric Fluid
SO	Surface Oil
TAG	Tri Acyl Glycerol
TG	Triglycerides
TLE	Total Life Expectancy
TO	Total Oil
USFDA	United States Food and Drug Administration
USP	US Pharmacopeia
VCO	Virgin Coconut Oil
WAC	Water Absorption Capacity
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Fatty acids, the fat-soluble components found in both animals and plants, are the primary building blocks of lipids and are classified as either saturated or unsaturated based on the presence of double bonds in their structure. Saturated fatty acids (SFAs) have no double bonds, while unsaturated fatty acids can be further divided into monounsaturated (MUFAs), containing one double bond, and polyunsaturated fatty acids (PUFAs), which have two or more double bonds (Sokoła-Wysoczańska et al., 2018; Islam et al., 2023). PUFAs are crucial for maintaining cell membrane integrity and are important in treating various conditions, including chronic illness, non-alcoholic fatty liver disease, and autoimmune disorders. Among PUFAs, essential fatty acids (EFAs) are a vital subclass that the human body cannot synthesize and must be obtained through the diet. EFAs are characterized by multiple double bonds and are classified into omega-3 and omega-6 fatty acids based on the position of the first double bond relative to the terminal methyl group in the aliphatic chain (Rizzo et al., 2023; Kapoor et al., 2021). Omega-3 fatty acids, such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), have their first double bond between the third and fourth carbon atoms from the methyl end, while omega-6 fatty acids, like linoleic acid (LA) and arachidonic acid (AA), have their first double bond between the sixth and seventh carbon atoms (Mariamenatu & Abdu, 2021). Omega-3 and omega-6 fatty acids provide significant health benefits, particularly for cardiovascular, inflammatory, and neurological health. EPA and DHA, lower triglyceride levels, improve blood flow, and support heart function, reducing the

risk of cardiovascular diseases. Their anti-inflammatory effects help manage conditions like arthritis and support mental health by reducing depressive symptoms and enhancing cognitive function. DHA is crucial for brain development, especially during pregnancy and early childhood. Meanwhile, omega-6 fatty acids, such as arachidonic acid, contribute to brain development and help lower LDL cholesterol, further promoting cardiovascular health (Djuricic & Calder, 2021).

The omega-6 to omega-3 ratio is a key indicator of the balance between these fatty acids, that is critical for overall health. However, modern Western diets often display a much higher ratio, sometimes exceeding 16:1, largely due to the prevalent consumption of foods rich in omega-6 fatty acids. The ideal ratio should range between 1:1 and 4:1. This imbalance in the ratio has significant health implications, as omega-6 fatty acids tend to promote inflammation, while omega-3s are known for their anti-inflammatory effects. Research highlights that excessive omega-6 intake, can exacerbate oxidative stress and inflammation, adversely affecting heart health and increasing the risk of chronic conditions such as diabetes, cardiovascular disease, and metabolic syndrome. Therefore, maintaining an appropriate balance by reducing the intake of omega 6 fatty acid and increasing the intake of omega 3 fatty acids can mitigate associated health risk (Brown et al., 2019; Djuricic & Calder, 2021; Jang & Park, 2020).

Seafood is often the best source of omega-3 fatty acids. Fishes known for their high omega-3 content include mackerel, herring, sardines, salmon, pollock, anchovies, and tuna (Oliver et al., 2020). Other sources of plant-based omega 3 fatty acids include nuts such as walnuts, seed oils such as garden cress, chia seed, inca inchi and flax seed,

seaweeds and microalgae (Saini et al., 2021; Kodahl et al., 2022; Rizzo et al., 2023). Fish oil is well known as the primary source of PUFAs. However, due to ecological, ethical, and taste-related factors, they are not consumed by everyone. However, PUFAs obtained from plant sources are beneficial over fish oil due to their neutral odour thus making it easier to develop omega fatty acid supplements in the form of chewable tablets as a better choice for consumers seeking alternatives to fish based products. Hence there is a need to identify plant-based sources that are globally acceptable and sustainable (Rizzo et al., 2023; Supriyanto et al., 2022).

1.2 Problem Statement

Inca Inchi Oil obtained from the seeds of inca inchi has been known to improve health due to the presence of PUFAs. However, the nutritional composition, antioxidant properties and quality of the oil depends on the extraction techniques such as Soxhlet extraction, supercritical CO₂ extraction, screw press extraction, aqueous enzyme assisted extraction etc. Moreover, inca inchi oil pressed cake, a byproduct obtained after extracting the inca inchi oil is known to contain functional ingredients such as protein, fiber, and minerals. However, the application of inca inchi oil press cake in bakery products is limited and the potential of developing oil into chewable oil tablets for delivering its health benefits has not been fully explored. Thus, this study aims to evaluate different extraction methods (microwave assisted extraction, dry roasting and aqueous enzyme assisted extraction) on the fatty acid composition, quality and antioxidant properties of inca inchi oil and to assess the feasibility of incorporating inca inchi oil and inca inchi oil press cake in chewable tablets and cookies.

1.3 Research Hypotheses

The study aims to address the following hypotheses

1. Microwave and dry roasting pre-treatments will significantly affect the yield, fatty acid composition, antioxidant properties, and overall quality of inca inchi oil.
2. Enzyme-assisted extraction of oil from inca inchi seeds can be optimized using response surface methodology, and the extracted oil will exhibit desirable characteristics.
3. Spray-dried microcapsules of oil blends (inca inchi oil-virgin coconut oil and inca inchi oil-red palm oil) will have high encapsulation efficiency, and the wall material will significantly influence the encapsulation efficiency.
4. Chewable tablets enriched with oil blend encapsulates can be successfully developed and characterized.
5. Partial replacement of whole wheat flour with inca inchi oil press cake in cookies will improve the physico-chemical and organoleptic properties of the cookies.

1.4 Research Objectives

The study aims to utilize inca inchi oil and its byproduct, press cake to develop chewable oil tablets from oil encapsulates and cookies from press cake. The following five objectives outlines the overall view of the research. The initial step involves investigating the effect of microwave and dry roasting pre-treatment on the oil yield, quality, antioxidant property and fatty acid composition. In the second part the study aims to optimize the extraction of inca inchi oil using enzymes such as Neutrase 0.8L and Viscozyme L by varying enzyme dose, hydrolysis time and incubation temperature to determine the extraction efficiency of the enzymes. These enzymes

were chosen based on their ability to break down the key components (proteins and cell wall components). Enzyme Neutrase 0.8 L is a protease enzyme that helps to break down the protein molecules surrounding the oil, facilitating the release of oil. Enzyme Viscozyme L is a multi-enzyme complex with various activities such as cellulase, hemicellulose, xylanase and beta-glucanase activities. They help to release the oil by breaking down the cell wall components such as cellulose, hemicellulose, pectin and β -glucans. Further the oil obtained were characterized for their quality, antioxidant property and fatty acid composition. In the third part of the study, two spray dried microcapsules of oil blend were developed by combining inca inchi oil with virgin coconut oil and red palm oil to assess the influence of total solids and inlet air temperature on the encapsulation efficiency. The oil encapsulates developed were used to develop chewable oil tablets as a convenient way of delivering the nutritional benefits of the oil blends. Finally, the research will investigate the incorporation of inca inchi oil press cake into cookie formulations by partially replacing whole wheat flour, and to assess the effect of partial replacement on the nutritional, physical and overall acceptability of the cookies.

Thus, the five objectives of the study were:

1. To determine the effect of microwave and dry roasting pre-treatments on the yield, fatty acid composition, antioxidant and quality of inca inchi seed oil.
2. To optimize the enzyme assisted extraction of oil from inca inchi seeds using response surface methodology and its characterization.
3. To evaluate the effect of process conditions and wall materials on the encapsulation efficiency of spray-dried microcapsules containing blends of inca inchi oil with virgin coconut oil or red palm oil.

4. To develop and characterize chewable tablets enriched with oil blend encapsulates
5. To evaluate the effect of partial replacement of whole wheat flour with inca inchi oil press cake in the formulation of cookies and to determine the physico chemical and organoleptic parameters of the cookies.



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