UNIVERSITI PUTRA MALAYSIA

EXTRACTION, CHARACTERIZATION AND STORAGE STABILITY OF OILS FROM SELECTED PLANT SEEDS

NYAM KAR LIN

FSTM 2009 25

EXTRACTION, CHARACTERIZATION AND STORAGE STABILITY OF OILS FROM SELECTED PLANT SEEDS

By

NYAM KAR LIN

Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Food Science and Technology Universiti Putra Malaysia

November 2009

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

EXTRACTION, CHARACTERIZATION AND STORAGE STABILITY OF OILS FROM SELECTED PLANT SEEDS

By

NYAM KAR LIN

November 2009

Chairman: Assoc. Prof. Dr. Tan Chin Ping, PhD

Faculty: Food Science and Technology

There is a great demand for renewable sources of raw materials that have nutritional and industrial potential. To meet the increasing demand for vegetable oils, improvements are being made with conventional crops as well as with selected plant species that have the ability to produce unique, desirable fats and oils.

The physicochemical properties and chemical composition of oil extracted from five varieties of plant seeds (bitter melon, Kalahari-melon, kenaf, pumpkin and roselle) were examined by established methods. Most of the quality indices and fatty acid compositions showed significant ($P < 0.05$) variations among the extracted oils. The oils were rich in tocopherols, with γ-tocopherol as the major component in all oil samples. Among the phytosterols, β-sitosterol was the major phytosterol extracted from the five plant-seed oils.

Enzymatic extraction of oil from Kalahari-melon seeds was investigated and evaluated by response surface methodology. Two commercial protease enzyme products were separately used: Neutrase® 0.8 L and Flavourzyme® 1000 L from Novozymes (Bagsvaerd, Denmark). Response surface methodology (RSM) was used to model and optimize the reaction conditions, namely concentration of enzyme (2-5 g/100 g of seed mass), initial pH of mixture (pH 5-9), incubation temperature (40-60 ˚C), and incubation times (12-36 h). The optimal conditions for Neutrase 0.8 L were enzyme concentration of 2.5 g/100 g, initial pH of 7, temperature at 58°C and incubation time of 31 h, yielding an oil recovery of 68.58 ± 3.39%. The optimal conditions for Flavourzyme 1000 L were: enzyme concentration of 2.1 g/100 g, initial pH of 6, temperature at 50 °C and incubation time of 36 h, yielding a 71.55 ± 1.28% oil recovery.

The physicochemical properties of oil from Kalahari-melon seed were determined following extraction with petroleum ether and aqueous-enzymatic methods. The free fatty acid, peroxide, iodine and saponification values of the oils extracted using these two methods were found to be significantly ($P < 0.05$) different. No significant ($P > 0.05$) difference was observed between the melting points of the oils obtained from solvent and aqueous-enzymatic extractions. Enzyme-extracted oil tended to be light-colored and more yellow in color, compared with solvent-extracted oil. Fatty acids and phenolic acids in enzyme-extracted oils were comparable to the solvent-extracted oil. The oils extracted with these two methods differed in the composition of their phytosterol and tocopherol contents, but no significant ($P > 0.05$) difference between the two enzyme-extracted oils was observed.

Supercritical carbon dioxide extraction of oil from Kalahari-melon and roselle-seeds were investigated in this study. Response surface methodology (RSM) was used to model and optimize the extraction conditions, namely pressure (200-400 bar), temperature (40-80 ºC) and supercritical fluid flow rate (10-20 mL/min). The optimal processing conditions for Kalahari-melon-seed oil recovery and phytosterol concentration were pressure of 300 bar, temperature of 40 °C and supercritical fluid flow rate of 12 mL/min. These optimal conditions yielded a 76.3% oil recovery and 836.5 mg/100 g of phytosterol concentration. The results indicate that the roselle-seed oil recovery was optimal, with a recovery of 102.61% and a phytosterol composition of 727 mg/100 g at the relatively low temperature of 40 ˚C, a high pressure of 400 bar and at a high supercritical fluid flow rate of 20 mL/min.

Tocopherol-enriched oil from Kalahari-melon and roselle-seeds was extracted by supercritical fluid extraction with carbon dioxide (SFE-CO_2). The optimal SFE-CO_2 conditions for the extraction of tocopherol-enriched oil from Kalahari-melon seeds were extraction pressure of 290 bar, extraction temperature of 58 ºC and flow rate of carbon dioxide of 20 mL/min. The optimum conditions for roselle-seeds were extraction pressure of 200 bar, extracting temperature of 80 ºC and flow rate of carbon dioxide of 20 mL/min. These optimum conditions yielded a tocopherol concentration of 274.74 and 89.75 mg/100 g oil from Kalahari-seed and roselle-seed, respectively.

During 6 months of storage of Kalahari-melon-seed and roselle-seed oils at both 4 ºC and room temperature in the darkness, changes occurred in the

content of fatty acids, phytosterols and tocopherols, and in the presence of primary and secondary oxidative products. These seed oils were obtained from the seeds of Kalahari melon (*Citrullus lanatus*) and roselle (*Hibiscus sabdariffa* Linn.) by supercritical carbon dioxide (SC-CO_2). As expected, statistically significant differences were observed in the content of fatty acids, phytosterols and tocopherols, and in the presence of primary and secondary oxidative products in Kalahari-melon-seed and roselle-seed oils throughout the storage. The quality indices peroxide and anisidine values increased during the 6 months storage time. After storage, degradation parameters may change because of lipid oxidation.

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

PENGESTRAKAN, PENCIRIAN DAN KESTABILAN PENYIMPANAN BAGI MINYAK DARIPADA BIJI BENIH TUMBUHAN TERTENTU

Oleh

NYAM KAR LIN

November 2009

Pengerusi: Prof. Madya Dr. Tan Chin Ping, PhD

Fakulti: Sains dan Teknologi Makanan

Sumber bahan mentah boleh dibaharui yang mempunyai potensi pemakanan dan perindustrian amat diperlukan. Untuk memenuhi permintaan yang semakin meningkat terhadap minyak-minyak sayuran, perbaikan telah dilakukan untuk tanaman lazim, begitu juga dengan spesis tumbuhan terpilih yang mempunyai kemampuan untuk menghasilkan lemak serta minyak yang unik dan diingini.

Sifat fiziko-kimia dan komposisi kimia bagi minyak yang diekstrak daripada lima jenis biji benih tumbuhan (peria, tembikai Kalahari, kenaf, labu dan roselle) dikaji dengan menggunakan kaedah yang telah ditetapkan. Kebanyakan indeks kualiti dan komposisi asid lemak menunjukkan variasi yang nyata ($P < 0.05$) antara minyak-minyak yang diekstrakkan. Minyak-minyak yang diekstrak kaya dengan tocoferol di mana γ-tocoferol merupakan komposisi yang

utama dalam minyak-minyak tersebut. β-sitosterol merupakan fitosterol yang utama dalam kelima-lima minyak biji tumbuhan.

Pengekstrakan minyak biji tembikai Kalahari dengan enzim dikaji dan dinilai dengan metodologi tindakbalas permukaan. Dua produk komersial enzim protease telah digunakan secara berasingan iaitu Neutrase® 0.8 L and Flavourzyme® 1000 L dari Novozymes (Bagsvaerd, Denmark). Metodologi tindakbalas permukaan telah digunakan untuk model dan keadaan reaksi bernama kepekatan enzim (2-5 g/100 g daripada berat biji), pH campuran awal (pH 5-9), suhu pengeraman (40-60 ˚C) dan tempoh pengeraman (12-36 h). Keadaan optimum bagi Neutrase® 0.8 L ialah kepekatan enzim 2.5 g/100 g , campuran awal pH 7, suhu pengeraman 58 ˚C dan tempoh pengeraman 31 jam dengan perolehan minyak sebanyak 68.58 ± 3.39%. Keadaan optimum bagi Flavourzyme® 1000 L ialah kepekatan enzim 2.1 g/100 g , campuran awal pH 6, suhu pengeraman 50 ˚C dan tempoh pengeraman 36 jam dengan perolehan minyak sebanyak 71.55 ± 1.28%.

Sifat fiziko-kimia minyak biji tembikai Kalahari yang diekstrak dengan kaedah petroleum eter dan enzim berair telah dikaji. Asid lemak bebas, nilai peroksida, iodin dan saponifikasi dalam minyak yang diekstrak dengan menggunakan kaedah-kaedah tersebut didapati berbeza dengan nyata ($P < 0.05$). Takat lebur minyak yang diekstrak dengan kaedah-kaedah tersebut didapati tiada perbezaan yang nyata ($P < 0.05$). Minyak yang diekstrak dengan enzim adalah lebih cerah dan warnanya lebih kuning daripada minyak yang diekstrak dengan pelarut. Asid lemak dan asid fenolik dalam minyak yang diesktrak dengan enzim adalah setanding dengan minyak yang diekstrak dengan pelarut. Minyak-minyak yang diekstrak dengan dua kaedah ini adalah berbeza dalam kandungan fitosterol dan tocoferol dari segi komposisi, tetapi tiada perbezaan yang nyata dalam kedua-dua minyak yang diekstrak dengan enzim.

Minyak-minyak biji tembikai Kalahari dan roselle yang diekstrak dengan supergenting karbon dioksida telah dikaji. Metodologi tindakbalas permukaan telah digunakan dalam model dan keadaan pengekstrakan dioptimumkan bernama tekanan (200-400 bar), suhu 40, 60 dan 80 ºC dan aliran cecair supergenting 10-20 mL/min. Keadaan proses yang optimum bagi perolehan minyak biji tembikai Kalahari dan kepekatan fitosterol ialah tekanan 300 bar, suhu operasi 40 °C dan aliran cecair supergenting 12 mL/min. Keadaan optimum ini dapat memperoleh 76.3% minyak biji tembikai Kalahari dan kepekatan fitosterol 836.5 mg/100 g. Keputusan menunjukkan bahawa perolehan minyak biji roselle adalah optimum dengan 102.61% dengan kehadiran komposisi fitosterol 727 mg/100 g dalam keadaan suhu yang rendah 40 ˚C, tekanan yang tinggi 400 bar dan aliran cecair supergenting yang tinggi 20 mL/min.

Minyak yang kaya dengan tocoferol telah diekstrak dengan pengekstrakkan cecair supergenting oleh karbon dioksida dari biji-biji tembikai Kalahari dan roselle. Keadaan optimum bagi pengekstrakkan minyak yang kaya dengan tocoferol dari biji tembikai Kalahari ialah tekanan pengekstrakkan 290 bar, suhu pengekstrakkan 58 ºC dan pengaliran karbon dioksida 20 mL/min. Keadaan optimum bagi pengekstrakkan minyak biji roselle adalah tekanan pengekstrakkan 200 bar, suhu pengekstrakkan 80 ºC dan pengaliran karbon dioksida 20 mL/min. Keadaan optimum ini memperoleh kepekatan tocoferol

274.74 dan 89.75 mg/100 g minyak daripada biji-biji tembikai Kalahari dan roselle masing-masing.

Semasa penyimpanan minyak-minyak biji tembikai Kalahari dan roselle selama 6 bulan pada suhu 4 ºC dan suhu bilik dalam kegelapan, perubahan berlaku dalam kandungan asid lemak, fitosterol, tocoferol, kehadiran produk pengoksidaan pertama dan kedua. Minyak-minyak tersebut adalah diperolehi daripada biji-biji tembikai Kalahari dan roselle dengan pengekstrakkan supergenting karbon dioksida. Seperti yang dijangkakan, perbezaan yang nyata dalam kandungan asid lemak, fitosterol, tocoferol, kehadiran produk pengoksidaan pertama dan kedua dalam minyak-minyak biji tembikai Kalahari dan roselle telah diperhatikan sepanjang penyimpanan. Kualiti indeks nilai peroksida dan anisidin telah meningkat semasa penyimpanan 6 bulan. Parameter degradasi mungkin berubah akibat pengoksidaan minyak selepas penyimpanan.

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest gratitude and respect to my kind supervisor, Associate Professor Dr. Tan Chin Ping for his understanding, guidance, encouragement, and support throughout my study. I would also like to extend my appreciation and gratitude to the members of the advisory committee Professor Dr. Yaakob Bin Che Man, Associate Professor Dr. Lai Oi Ming and Dr. Kamariah Long for their invaluable contributions and support.

My sincere gratitude is also extended to the financial support provided by the Science Fund for this research, which was awarded to Associate Professor Dr. Tan Chin Ping. I am also indebted to all the staff of the Faculty of Food Science and Technology for their generous cooperation. Acknowledgement is also due to all my colleagues and laboratory assistants who had given me the moral encouragement and support to complete my graduate study.

Last but not the least, I also wish to express my deepest appreciation to my beloved parents, elder brother and younger brothers who have given me encouragement and support in one way or another during the many years of my seemingly never ending pursue for knowledge.

I certify that an Examination Committee has met on 16 November 2009 to conduct the final examination of Nyam Kar Lin on her Doctor of Philosophy thesis entitled "Extraction, Characterization and Storage Stability of Oils from Selected Plant Seeds" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Examination Committee were as follows:

Mohd Yazid Manap, Ph.D.,
Professor,
Faculty of Food Science and Technology,
Universiti Putra Malaysia.
(Chairman)

Md Zaidul Islam Sarker, Ph.D.,
Associate Professor,
Faculty of Food Science and Technology,
Universiti Putra Malaysia.
(Internal Examiner)

Lasekan Olusegun, Ph.D.,
Associate Professor,
Faculty of Food Science and Technology,
Universiti Putra Malaysia.
(Internal Examiner)

David B Min, Ph.D.,
Professor,
Department of Food Science and Technology,
The Ohio State University,
Columbus.
(External Examiner)

BUJANG KIM HUAT, PH.D.,
Professor and Deputy Dean
School of Graduate Studies,
Universiti Putra Malaysia

Date:

The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy; the members of the Supervisory Committee were as follows:

Tan Chin Ping, PhD
Associate Professor,
Faculty of Food Science and Technology,
Universiti Putra Malaysia.
(Chairman)

Yaakob Bin Che Man, PhD
Professor,
Faculty of Food Science and Technology,
Universiti Putra Malaysia.
(Member)

Lai Oi Ming, PhD
Associate Professor,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia.
(Member)

Kamariah Long, PhD
Doctor,
Malaysian Agricultural Research & Development Institute (MARDI)
(Member)

HASANAH MOHD GHAZALI, PHD
Professor and Dean
School of Graduate Studies,
Universiti Putra Malaysia

Date: 14 January 2010

Declaration Form

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NYAM KAR LIN
Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

GENERAL INTRODUCTION

Recently, more attention has been focused on the utilization of food processing by-products and wastes, as well as under-utilized agricultural products. Obviously, such utilization would contribute to maximizing available resources and result in the production of various new foods. Simultaneously, waste disposal problems could be minimized.

The problems of industrial waste are becoming harder to solve, and much effort will be needed to develop the nutritional and industrial potential of by-products, waste and under-utilized agricultural products. Only a small portion of plant material is utilized directly for human consumption (El-Adawy *et al.*, 1999). A portion of the remaining material may be converted into nutrients for either food or feed, or into fertilizer, making possible an important contribution to food resources or industrial products (El-Adawy *et al.*, 1999; Kamel *et al.*, 1982). For example, the seeds of the bitter melon, Kalahari-melon, kenaf, pumpkin and roselle could be used; these seeds are present in large quantities as waste products after the removal of the pulp, peel and flesh of these plants.

Bitter melon (*Momordica charantia* L.), also known as bitter gourd, is a monoecious climbing vine. It is a tropical crop, grown throughout Asia for food and medicinals (Chakravarty, 1990). The seeds contain oil in which the major fatty acid is eleostearic acid (ESA), which is a major component of oil from tung nuts and is the constituent responsible for the "drying" characteristic of tung oil. The latter is used extensively in paints, coatings and inks.

Kalahari-melon (*Citrullus lanatus*) is the most important source of water in the Kalahari during dry months of the year when no surface water is available. The fruit is cut open at the one end and the first piece of flesh is eaten. The remaining contents are pounded with a stick, and are then eaten and drunk. Seeds are roasted and ground into meal—a nutritious food with a pleasant, nutty taste. The leaves and young fruit are utilized as green vegetables (Van Wyk and Gericke, 2000). The peels of the fruit are traditionally used for making jam. The cultivated watermelon is a popular summer fruit in all parts of the world.

Kenaf (*Hibiscus cannabinus* L.) is a warm season annual belonging to the Malvaceae family, which also includes cotton (*Gossypium* spp.) and okra (*Abelmoschus esculentus* L. Moench). It has been used for thousands of years in Africa and parts of Asia as a source of fiber for making clothes, rugs, ropes and other product. The commercial uses of kenaf continues to diversify from its historical role as cordage to its various new applications, including paper products, building materials, absorbents and livestock feeds (Webber and Bledsoe, 1993; Sullivan, 2003). Seeds from kenaf fruit may provide an excellent oil resource. The oil has chemical