UNIVERSITI PUTRA MALAYSIA

COCOA BUTTER EXTRACTION FROM COCOA NIBS USING SUPERCRITICAL CARBON DIOXIDE

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COCOA BUTTER EXTRACTION FROM COCOA NIBS USING SUPERCRITICAL CARBON DIOXIDE

By
ASEP EDI KUSNADI

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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This Thesis is specially dedicated to

Mamah and Apa (alayarham), Aih, Ujang, Dadan and Nanang and all their family for the unconditional patient, love and support.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

COCOA BUTTER EXTRACTION FROM COCOA NIBS USING SUPERCRITICAL CARBON DIOXIDE

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Chairman: Professor Jinap Selamat, PhD

Faculty: Food Science and Technology

Cocoa beans consist mainly of cocoa butter (50-55% w/w). High quality cocoa butter used in food, cosmetic and pharmaceutical products is obtained by mechanical press, expeller, and solvent extraction using hexane. Supercritical fluid extraction (SFE) using carbon dioxide as a solvent has provided an excellent alternative to the use of chemical solvent in the extraction of cocoa butter from different plant matrices. In comparison with established methods, SFE has some important advantages, particularly in its ability to yield products that are completely free from processing residues. SFE is also an alternative from the standpoint of time-saving and for environmental reasons such as the reduction of the use of large solvent volumes. Carbon dioxide (CO_2) is an ideal solvent for extraction of natural products because it is nontoxic, odorless, tasteless, non explosive, readily available, and easily removable from the products.

This work is divided into several part namely; 1) the effect of flow rate with different pressure on cocoa butter extraction using supercritical carbon dioxide ($SC\text{-}CO_2$), 2) a study on the sample matrix involving: the effect of particle size, degree of fermentation and alkalization on cocoa butter extraction using supercritical fluid; 3) a study on the effect of moisture content, roasting time and roasting temperature on cocoa butter extraction using supercritical fluid, 4) a study on the effect of cosolvents with types and concentrations of polar cosolvents and non polar cosolvents in cocoa butter extraction using $SC\text{-}CO_2$, 5) to evaluate the mass transfer parameters in cocoa butter extraction by SFE using the Sovova Lacks plug flow model (SLM) for the effect of flow rate with different pressure, the effects of polar and non polar cosolvents, and also using single sphere model (SSM) for the effects of particle size, degree of fermentation, pH alkalization, moisture content, roasting time and roasting temperature and 6) to determine the triacylglycerols composition and fatty acid methyl esters composition of cocoa butter extracted that resulted from extraction using $SC\text{-}CO_2$ and $SC\text{-}CO_2$ with cosolvents.

The study on the effect of flow rate and pressure found that the optimum conditions for flow rate and pressure for cocoa butter extraction using $SC\text{-}CO_2$ were at 2 ml/min and 35 MPa, r espectively. The highest yields were obtained from cocoa nibs sample with smaller particle size (S1= 0.07 mm) with 92% yield for almost 20 h using $SC\text{-}CO_2$, unfermented nibs (F1) with 100% yield for almost 10 h using ethanol 25% as cosolvent in $SC\text{-}CO_2$ and roasting treatment of 150 ^{o}C and 35 min (R6) with 100% yield for almost 14 h using ethanol 25% (mol %) as cosolvent in $SC\text{-}CO_2$. Increasing roasting time and temperature have resulted in the increase of the yield. Furthermore,

the highest yield was produced from high pH (7.5 -7.9) of dark alkalized cocoa liquor (A1) with 73.70% yield for nearly 18 h extraction time using ethanol 25% as cosolvent in SC-CO_2 and high moisture content (M5 =17.64%) with 60.73% after nearly 20 h extraction using SC-CO_2.

Statistically, the yield (p<0.05) significantly was affected by all the treatment, however no significant different was observed for both light alkalized cocoa liquor (pH = 6.8-7.2) and alkalized natural cocoa liquor (pH = 5.0 – 5.9), and both moisture content of 9.79 and 17.64%, respectively.

Ethanol showed the best cosolvent on the yield in SC-CO_2, followed by isopropanol, acetone, hexane and cyclohexane. The yield increased with an increase in concentration of cosolvents (25%>15%>5%), except for acetone in which 15% concentration was higher than 25% and 5%. Extraction of cocoa butter using SC-CO_2 with polar cosolvents obtained a yield higher than non polar cosolvents. All treatment of cosolvents studied statistically significantly different at p<0.05.

The two mathematical models are based on mass transfer were used. First model, the Sovova's lack's plug flow model (SLM) was used to describe the extraction process of effect of flow rate with different pressure and effect of polar and non polar cosolvents. The hardly accessible solute xk and the volume mass transfer coefficients in the fluid phase (F) and solid phase S were used as fitting parameters. The maximum average deviation between measured and calculated oil yield was 6.861%. Mass transfer coefficients in the fluid phase and solid phase varied between 6.528.E-

06 to 1.498E-04 s^{-1} and between 5.185.E-06 to 9.144E-04 s^{-1}, respectively. Second model, the single sphere model (SSM) was used to describe the extraction process of effect of particle size, degree of fermentation, pH of alkalization, moisture content, roasting time and roasting temperature. Adjusting of effective intraparticle diffusion coefficient (*De*) and estimation of parameter of coefficient of mass transfer in the fluid phase (*kf*) and overall mass transfer coefficient *kp* and parameters were evaluated. The result showed that *De, kf* and *kp* varied between 8.900E-16 to 1.850E-09 m^2/s, between 1.139E-10 to 2.115E-04 m/s and between 9.581E-12 to 8.992E-05 m/s, respectively. All experimental data results of extraction curves were fitted fairly well by using both of the SLM model and SSM model with average absolute relative deviation (AARD) between measured and calculated oil yield were maximum of 6.861.

The results showed that cocoa butter extracted from various treatments had three main triacylglycerol (TG) namely Glycerol-1,3-Dipalmitate-2-Oleate (POP), Glycerol-1-Palmitate-2-Oleate-3-Stearate (POS), and Glycerol-1,3-Distearate-2-Oleate (SOS). In general, POS was the major component with 42.16 to 45.78% followed by SOS and POP with 27.60 to 31.67% and 20.09 to 22.79%, respectively. Furthermore, analysis of fatty acid (FA) showed that palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) were the three main fatty acids in the cocoa butter extracted with stearic acid being the major component with 34.82 to 39.06%, followed by oleic acid and palmitic acid with 28.48 to 31.72% and 28.27 to 31.33%, respectively. Statistically, there are differences the effects of all treatment on TG and FA compositions.

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

PENGEKSTRAKAN LEMAK KOKO DARI PADA NIB KOKO MENGGUNAKAN BENDALIR SUPERKRITIK KARBON DIOKSIDA

Oleh

ASEP EDI KUSNADI

April 2009

Pengerusi: Profesor Jinap Selamat, PhD

Fakulti: Sains dan Teknologi Makanan

Sebahagian besar daripada komponen biji koko terdiri daripada lemak koko (50-55%), dimana lemak koko yang berkualiti tinggi yang digunakan di dalam produk makanan, kosmetik dan farmaseutikal diperolehi melalui proses pengekstrakan menggunakan tekanan secara mekanik, penggunaan tekanan dengan kuasa putaran dan pelarut kimia seperti heksana. Proses pengekstrakan dengan cecair superkritik (SFE) yang menggunakan gas karbon dioksida sebagai pelarut telah menjadi satu alternatif yang baik bagi pengekstrakan lemak koko daripada matriks tumbuhan yang berbeza berbanding pengekstrakan menggunakan pelarut kimia. Dibandingkan dengan cara yang sedia pakai, SFE mempunyai beberapa kelebihan terutamanya dalam menghasilkan produk yang bebas dari sisa-sisa pemprosesan. SFE juga lebih baik dari segi penjimatan masa dan lebih mesra alam sekitar kerana mengurangkan penggunaan pelarut dalam isi padu yang tinggi. Karbon dioksida pula merupakan pelarut yang

ideal bagi pengekstrakan produk asli kerana ciri-cirinya yang tidak toksik, tidak berbau, tidak mempunyai rasa, tidak mudah meletup/terbakar, sedia didapati dan mudah dipisahkan dari produk.

Kajian ini dimulakakan dengan mengkaji, 1) kesan kadar alir dengan tekanan yang berbeza ke atas pengekstrakan lemak koko menggunakan supercritical karbon dioksida (SC-CO_2), 2) kajian matriks sampel: kesan saiz partikel, darjah penapaian dan pengalkalian, 3) kajian untuk kesan kandungan kelembapan, masa pemanggangan dan suhu pemanggangan dan 4) kesan pelarut penyokong/pengubahsuai (CS) mengikut jenis dan kepekatan dari pada CS yang bersifat polar dan tidak polar semasa pengekstrakan lemak koko menggunaan SC-CO_2, 5) kajian pemindahan jisim menggunakan model Sovova yang berasaskan model lanjutan aliran palam Lack (SLM) untuk pengaruh kadar alir dengan tekanan berbeza dan kesan CS yang polar dan tak polar, dan juga model partikel bulat tunggal (SSM) untuk pengaruh saiz partikel, tingkatan penapaian, pengalkalian, massa pemanggangan dan suhu pemanggangan dan 6) kajian komposisi daripada trigliserida dan asid lemak yang terekstrak menggunakan SC-CO_2 dan CS.

Kajian ini mendapati keadaan optimum bagi kadar alir dan tekanan yang digunakan untuk pengekstrakan lemak koko menggunakan SC-CO_2 adalah pada 2 mL/min dan 35 MPa. Hasil tertinggi diperolehi daripada nib koko yang mempunyai saiz partikel terkecil (S1= 0.07 mm) dengan penghasilan 92% setelah hampir 20 jam menggunakan SC-CO_2, nib koko tertapai (F1) dengan penghasilan 100% setelah hampir 10 jam menggunakan etanol sebagai CS di dalam SC-CO_2 dan juga bagi pemanggangan 150

^{o}C dan 35 min (R6) dengan 100% hasil setelah hampir 14 jam menggunakan 25% etanol (%mol) sebagai CS di dalam SC-CO_2. Peningkatan suhu dan masa pemanggangan telah menningkatkan hasil yang diperolehi. Hasil yang tinggi juga diperolehi daripada likur koko hitam yang beralkali pada pH tinggi 7.5-7.9 (A1) dengan 73.70% hasil setelah pengekstrakan selama 18 jam menggunakan 25% etanol sebagai CS di dalam SC-CO_2 dan juga pada kandungan kelembapan yang tinggi (M5 = 17.64%) dengan 60.73% hasil setelah pengekstrakan menggunakan SC-CO_2 selama 20 jam.

Secara statistiknya, hasil yang diperolehi dipengaruhi oleh semua perlakuan penyelidikan pada $p<0.05$, tetapi tiada perbezaan yang signifikan diperolehi bagi kedua-dua koko likur beralkali rendah (pH = 6.8-7.2) dan koko likur beralkali asli (pH = 5.0 – 5.9) dan juga pada kedua-dua kandungan kelambapan masing-masing 9.79 dan 17.64%.

Etanol telah menunjukkan ciri terbaik sebagai CS bersama SC-CO_2, diikuti oleh isopropanol, aseton, siklohesana dan heksana. Peningkatan hasil diperolehi bersama peningkatan kepekatan (mol%) daripada CS (25%>15%>5%), kecuali bagi aseton yang mana pada kepekatan 15% mempunyai hasil yang lebih tinggi dari 25 dan 5%. Pengekstrakan lemak koko dengan SC-CO_2 bersama CS yang polar mempunyai hasil yang tinggi berbanding CS yang tidak polar. Semua perlakuan dalam penyelidikan tentang pengaruh CS secara statistik menunjukkan perbezaan yang signifikan pada $p<0.05$.

Kedua dua model berasaskan pemindahan jisim dapat dilakukan. SLM dapat digunakan untuk menjelaskan terjadinya pengaruh kadar alir dengan tekanan berbeza dan efek dari pada jenis dan konsentrasi CS yang polar dan non polar. Parameter dari *xk, F* dan *S* dapat digunakan untuk penilaian parameter pemindahan jisim dalam fasa bendalir dan padatan dari pada data eksperimen. AARD maksimum adalah 6.861%. Koefisien pemindahan jisim fasa bendalir dengan padatan adalah beragam masing-masing diantara 6.528.E-06 - 1.498E-04 s^{-1} dan 5.185.E-06 - 9.144E-04 s^{-1}. Untuk model kedua (SSM), hasil kajian menunjukkan bahawa nilai-nilai *De, kf dan kp* beragam, dimana masing-masing berada diantara 8.900E-16 - 1.850E-09 m^2/s, 1.139E-10 - 2.115E-04 m/s dan 9.581E-12 - 8.992E-05 m/s. Semua rajah dari data eksperimen memberikan hasil yang bersesuaian dengan model, dengan AARD maksimum pada 6.861.

Keputusan menunjukkan lemak koko yang diekstrak dari proses yang berbeza mengandungi tiga jenis trigliserida (TG) utama yaitu 1,3-Dipalmitoyl-2-Oleoyl-Gglycerol (POP), 1-Palmitoyl-2-Oleoyl-3-Stearoyl-Glycerol (POS) dan 1,3-Distearoyl-2-Oleoyl-Glycerol (SOS). Secara amnya, POS didapati dalam kandungan yang tertinggi sebanyak 42.16-45.78% diikuti oleh SOS dan POP masing-masing sebanyak 27.60-31.67% dan 20.09-22.79%. Analisa asid lemak (FA) menunjukkan bahawa asid palmitik (C16:0), asid stearik (C18:0) dan asid oleik (C18:1) merupakan tiga asid lemak utama didalam lemak koko yang diekstrak dengan kandungan tertinggi asid strearik sebanyak 34.82-39.06%, diikuti oleh asid oleik dan asid palmitik masing-masing sebanyak 28.48-31.72 dan 28.27-31.33%. Secara statistik terdapt pengaruh yang berbeda dari semua perlakukan terhadap kandungan TG dan FA lemak.

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I certify that a Thesis Examination Committee has met on 28 April 2009 to conduct the final examination of Asep Edi Kusnadi on his thesis entitled "Cocoa Butter Extraction from Cocoa Nibs Using Supercritical Carbon Dioxide" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Pertanian Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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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 degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Date: 16 October 2009

DECLARATION

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 institutions.

ASEP EDI KUSNADI

Date: 15 September 2009

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