UNIVERSITI PUTRA MALAYSIA

FRACTIONAL EXTRACTION OF COCONUT OIL BY SUPERCRITICAL FLUID EXTRACTION AND ITS APPLICATION AS COCOA BUTTER SUBSTITUTE IN CHOCOLATE PRODUCTION

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FSTM 2018 13

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Cocoa butter is generally produced from coconut oil (CNO) and palm kernel oil which has similar physical characteristics as cocoa butter (CB). However, CNO contains high lauric acid which is different from CB and can be reduced by fractional extraction using Supercritical Fluid extraction (SFE). Therefore, aim of this study was to produce CBS from coconut oil (CNO) by fractional extraction with Supercritical Fluid Extraction (SFE). The objectives of this study were: i) to determine the effects of different pressures and temperatures on extraction of CNO and CNO fractions by SFE, ii) to determine the effects of different level of coconut oil fractions substituted CB in milk chocolate. CNO was extracted with SFE at four different pressures (27.6, 34.5, 41.4, 48.3MPa) and temperatures of 60 ºC and 80 ºC. Then, the selected parameter conditions were applied for fractional extraction of CNO with SFE. The selected F-CNO was applied as CBS in milk chocolate production at different level. The properties of milk chocolates which include melting profile, hardness, rheology, consumer acceptance and bloom stability were analysed to determine the effects of F-CNO substitute in milk chocolate.

The yield of CNO extracted at the highest pressure, 48.3 MPa and temperature, 80 ºC was significantly ($p<0.05$) higher (63.1%) and significantly ($p<0.05$) lower lauric acid (44.75%) compared to others. The pressure at 48.3 MPa and temperature of 80 ºC was selected as SFE operating parameter for the fractional extraction of the CNO. The fractional extraction give four different fractions, F1, F2, F3 and F4. F1 had significantly highest ($p<0.05$) yield (48.9%). The F4 contained significantly ($p<0.05$) lowest short and medium chain fatty acids while highest long chain fatty acids compared to F1, F2 and F3. F4 also had significantly ($p<0.05$) highest Tonset (24.2 ºC) and Tendset (33.6 ºC) which were close to CB. The solid fat content of F4 was 0% at temperature between 35 ºC to 40 ºC. Therefore, F4 was selected as the potential fraction of CNO (F-CNO) to be applied as CBS due to close properties to CB. The F-CNO (F4) was applied in milk chocolate to substitute cocoa butter at 1.5% (chocolate A), 3.0% (chocolate B) and 4.5% (chocolate C) compared with control chocolate (without F-CNO). The different levels of F-CNO substituted in milk chocolate resulted in different triglyceride composition which affected the melting profile, hardness and rheology. The melting profile of the milk chocolates A, B and C decreased significantly ($p<0.05$) as level of F-CNO added increased. The initial melting point of the milk chocolate decreased from 30.72 ºC to 28.98 ºC while the end melting point decreased significantly from 40.31 ºC to 36.36 ºC. The hardness of the milk chocolate significantly ($p<0.05$) decreased from control, followed by chocolate A, then, chocolate B and finally chocolate C. The rheology of the milk chocolate decreased significantly ($p<0.05$) when level of F-CNO added increased. Sensory evaluation shown consumer panelists (52%) significantly most preferred ($p<0.05$) the chocolate C which contain 4.5% F-CNO in terms of glossiness (6.72) taste, (6.92) and overall acceptability (6.60) compared to control (glossiness=5.57, taste=5.70 and overall=5.57). During bloom stability study, the whiteness index (WI) of control chocolate was higher compared to chocolate with F-CNO. Based on these findings, the F-CNO has the potential to be applied as CBS in chocolate based product with high stability.

Key words: fractional extraction, supercritical fluid extraction, coconut oil, coconut oil fraction, cocoa butter substitute, milk chocolate

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGEKSTRAKAN PECAHAN MINYAK KELAPA MENGGUNAKAN PENGEKSTRAKAN SUPERKRITIKAL BENDALIR DAN KEGUNAANNYA SEBAGAI LEMAK GANTIAN KOKO

Oleh

HALIMATUN SA'ADIAH BINTI ABDUL HALIM

Mei 2017

Pengerusi : Profesor Madya Norhayati Hussain, PhD
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Lemak koko gantian (CBS) yang didapati daripada minyak kelapa (CNO) dan isirung kelapa sawit mempunyai ciri-ciri fizikal kimia yang menyamai CB. Tetapi, minyak kelapa mempunyai jumlah asid lemak laurik yang tinggi di mana berbeza dari CB dan dapat dikurangkan melalui proses pengekstrakan pecahan menggunakan Pengekstrakan Superkritikal Bendalir (SFE). Oleh itu, kajian ini bertujuan menghasilkan CBS daripada CNO dengan cara pengekstrakan pecahan menggunakan SFE. Objektif kajian ini adalah: i) untuk mengkaji kesan perbezaan tekanan dan suhu terhadap pengekstrakan CNO dan pecahan CNO dengan, ii) untuk mengkaji kesan kadar pecahan CNO (F-CNO) yang berbeza menggantikan CB terhadap coklat susu. CNO diekstrak oleh SFE menggunakan empat tekanan yang berbeza (27.6, 34.5, 41.4, 48.3MPa) dan suhu (60 ºC dan 80ºC). Kemudian, parameter tekanan dan suhu yang terpilih diaplikasi untuk pengekstrakan pecahan CNO oleh SFE. Pecahan yang terpilih diaplikasikan sebagai CBS di dalam penghasilan coklat susu dengan kadar kuantiti yang berbeza. Ciri-ciri takat lebur, tahap kekerasan, reologi, penerimaan konsumer dan kestabilan bebunga lemak telah dianalisa untuk menentukan kesan gantian F-CNO di dalam coklat susu.

CNO yang diekstrak pada tekanan dan suhu tertinggi, 48.3 MPa dan 80 ºC menunjukkan hasil yang signifikan ($p<0.05$) paling tinggi (63.1%) dan kandungan asid laurik yang signifikan ($p<0.05$) paling rendah (44.75%) berbanding ekstrak CNO yang lain. Oleh itu, tekanan pada 48.3 MPa dan suhu pada 80 ºC ini telah dipilih sebagai operasi parameter SFE dalam pengekstrakan pecahan CNO. CNO telah diekstrak kepada empat pecahan yang berbeza, F1, F2, F3 dan F4. F1 mempunyai hasil CNO yang signifikan tinggi ($p<0.05$) dengan jumlah 48.9%. F4 mengandungi kandungan asid lemak rantaian pendek dan sederhana yang signifikan rendah ($p<0.05$) dan asid lemak rantaian panjang yang tinggi berbanding pecahan F1, F2 dan F3. F4 juga menunjukkan signifikan tertinggi ($p<0.05$) pada bacaan Tonset (24.24ºC) dan Tendset (33.64ºC) yang menghampiri CB. Kandungan pepejal lemak F4 adalah 0% pada suhu antara 35 ºC hingga 40 ºC. Oleh itu, F4 telah dipilih sebagai pecahan minyak kelapa (F-CNO) yang paling berpotensi sebagai CBS kerana mempunyai ciri yang paling hampir dengan CB. F-CNO (F4) telah diaplikasikan di dalam penghasilan coklat susu menggantikan kandungan CB pada 1.5% (coklat A), 3.0% (coklat B) dan 4.5% (coklat C) dan dibandingkan dengan coklat kawalan (tanpa F-CNO). Jumlah F-CNO yang berbeza menggantikan CB di dalam coklat susu menyebabkan komposisi trigliserida (TAG) yang berbeza dan memberikan kesan pada profil lebur, kekerasan dan reologi. Profil lebur coklat susu menurun secara signifikan ($p<0.05$) apabila kandungan F-CNO menggantikan CB meningkat. Nilai Tonset dan Tendset masing-masing menurun daripada 30.72 ºC ke 28.98 ºC dan daripada 40.31 ºC ke 36.36 ºC megikut turutan. Kekerasan coklat susu menurun secara signifikan ($p<0.05$) dari coklat susu kawalan, diikuti, coklat A, kemudian coklat B dan akhir sekali coklat C. Reologi coklat susu menurun dengan signifikan ($p<0.05$) apabila jumlah F-CNO yang ditambah meningkat. Penilaian deria rasa menunjukkan kebanyakan ahli panel (52%) signifikan ($p<0.05$) memilih coklat C yang mengandungi 4.5% F-CNO pada skor kekilatan (6.72), rasa, (6.92) dan penerimaan keseluruhan (6.60) yang tertinggi berbanding dengan coklat kawalan tanpa F-CNO (kekilatan = 5.57, rasa = 5.70 dan keseluruhan = 5.57). Kajian bebunga lemak menunjukkan indeks keputihan (WI) coklat kawalan adalah lebih tinggi berbanding coklat yang mengandungi F-CNO. Berdasarkan hasil yang diperoleh, F-CNO mempunyai potensi sebagai CBS bagi menggantikan lemak koko dalam produk berasaskan coklat dengan kestabilan yang tinggi.

Kata kunci: pengekstrakan pecahan, pengekstrakan superkritikal bendalir, minyak kelapa, pecahan minyak kelapa, lemak gantian koko, coklat susu

ACKNOWLEDGEMENT

Alhamdulillah, praise to Allah s.w.t., finally, I managed to complete my master project after two years of struggle and hard work. First and foremost I am grateful and would like to express my sincere gratitude to my supervisor, Assoc. Prof. Dr. Norhayati binti Hussain, whose patient guidance, dedicated involvement, useful critiques and deep knowledge in their fields have shown and guided me throughout the process of completing my project.Without her advice and assistance it would be hard for me to complete this study. I would like to express my warm thanks to my co-supervisors, Prof Jinap binti Selamat and Assoc. Prof. Dr. Sayed Hamed Mirhessoini for their support, encouragement and guidance throughout my study.

Special thanks to my parents, Mr Abdul Halim Ahmad and Mrs Amnah Mohd Saad whose endearing love has provided me with endless moral and financial support. I am really thankful for your sacrifice, patience, and understanding for all this time. Thanks to all my family members who always support me during my hard time.

I would also like to show gratitude to the staff of the Malaysian Cocoa Board, Nilai, Negeri Sembilan; processing laboratory and biochemical laboratory, Faculty of Food Science and Technology, UPM, Selangor for their kind assistance and their permission in using the laboratory facilities. I also wish to acknowledge the help I have received from my friends, especially Nurul Husna, Fara Syazana, Aya Putri, Roiaini, and Aisyah Zafirah for accompanying me in the laboratory and whose helps and gives support to me.

I certify that a Thesis Examination Committee has met on 15 May 2017 to conduct the final examination of Halimatun Sa'adiah binti Abdul Halim on her thesis entitled "Fractional Extraction of Coconut Oil by Supercritical Fluid Extraction and its Application as Cocoa Butter Substitute in Chocolate Production" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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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 the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

°C	Degree celcius
µL	micro Litre
µm	micro metre
CB	Cocoa butter
CBA	Cocoa butter alternative
CBE	Cocoa butter equivalent
CBR	Cocoa butter replacer
CBS	Cocoa butter substitute
CNO	Coconut oil
CO_2	Carbon dioxide
F-CNO	coconut oil fraction
h	hour
g	gram
kg	kilo gram
m	metre
mm	millimetre
min	minute
mL	milli Litre
MPa	Milli Pascal
N	Newton
Pa	Pascal
Pa.s	Pascal per second
POP	1,3-dipalmitoyl-2-oleoyl-glycerol
POS	1-palmitoyl-3-stearoyl-2-oleoyl-glycerol
SOS	1,3-distearoyl-2-oleoyl-glycerol
SFE	Supercritical fluid extraction
SFC	Solid fat content
TAG	Triglyceride
WI	Whiteness Index

CHAPTER 1

INTRODUCTION

The predominant raw material used in manufacturing of chocolate is cocoa butter (CB) (Steinberg, 2003). Cocoa butter is obtained from the seed of *Theobroma cacao* plant which is mainly cultivated in Ivory Coast, Ghana, Indonesia, Cameroon, Nigeria, Brazil and Malaysia, and contains 80% monounsaturated triglycerides and stable β crystals which give unique characteristics to chocolate with good snap, rapidly melt at body temperature and smooth mouth feel (Lipp et al., 2001). Despite that, there is an issue on limited supply of CB in the leading producer countries due to weather, pests and political factors (Medeiros & Lannes, 2009). Coupled with the fact that the price of CB is increasing annually, food manufacturers are therefore developing alternatives for future applications (Jahurul et al., 2013). According to the International Cocoa Organization, the price of CB in June 2013 was USD 2,283.58 per ton and increased to USD 3,122.52 per ton in June 2016 (ICCO, 2016).

According to Afoakwa et al. (2007), there are several vegetable fats that possess similar physical and chemical properties to those of CB. Therefore, these fats can be added in chocolate production to substitute CB. However, the amount of these fats to be used also depends on their physical and chemical properties (Talbot, 2009). At present, numerous studies have been conducted in the search for CB alternatives including cocoa butter equivalent (CBE), cocoa butter replacer (CBR), and cocoa butter substitute (CBS) from various vegetable fats such as kokum fat (Reddy & Prabhakar, 1994), beef tallow (Osborn & Akoh, 2005), palm kernel oil (Calliauw et al., 2005), palm oil (Pinyaphong & Phutrakul, 2009) and mango seed fat (Jahurul et al., 2014).

Palm kernel (PKO) and coconut oils (CNO) have similar characteristics to CB such as hardness, mouth feel and flavor release and therefore have been applied as CBS. According to EU Directive 2000/36/EC, CBS is allowed to be incorporated in the production of chocolate together with CB at a limit below 5%. There are several reports available on the production of CBS from PKO (Zaidul et al., 2006; Calliauw et al., 2005; Norulaini et al., 2004). However, to the best of our knowledge, the reports on the production of (CNO) as CBS are highly scarce.

Coconut oil is obtained from the dried coconut meat, known as copra. Commonly, CNO is extracted via pressed method with an expeller. It has a high degree of saturation and stability due to its fatty acid composition (Krishna et al., 2010). It is the desired type of oil for confectionery formulations and has been applied in confectionery products like nondairy/imitation dairy products, coffee whiteners and biscuit-filler creams (Pantzaris & Basiron, 2002). In the production of chocolate, Indarti et al. (2013) has used virgin CNO as CBS to improve the appearance and lower the rates of bloom formation in chocolate bar.

However, CNO contains high level of lauric acid which is significantly different from CB. The difference between these fatty acids causes the incompatibility when CNO is mixed with CB (Talbot, 2009). In addition, when CNO is exposed to moisture, lipase will cleave the fats and liberate the lauric acid which has distinct soapy flavor (Shukla, 2005; Tanabe & Hofberger, 2005; Minifie; 1989).

At present, there are several researches that have been conducted to investigate the reduction of lauric acid amount by fractionation using Supercritical Fluid Extraction (SFE) with carbon dioxide (CO_2). Norulaini et al. (2004) fractionated PKO with SFE and successfully reduced the amount of lauric acid from 53.65% to 31.86%. They reported that the fraction with the lowest lauric acid was blended together with stearic and oleic acids to produce CBE. In a later study, Zaidul et al. (2006) have also successfully reduced the amount of lauric acid from 60.09% to 28.11% in the fractionation of palm kernel. They concluded that the fraction with the lowest amount of lauric acid has the potential for the production of CBR.

According to Sahena et al. (2009), the fractionation of fats by SFE with carbon dioxide (CO_2) has gained much attention. The fractionation method alters the solubility of fatty acids by changing the pressure and temperature of the SFE operating conditions. Therefore, the fats are fractionated into different melting fractions based on the fatty acid compositions. The products obtained are separated into short, medium and long chain fatty acids (Buyukbese et al., 2014). There are several researches that have used SFE with CO_2 to modify the fats for further applications which include PKO (Zaidul et al., 2006; Hassan et al., 2000), buffalo butter oil (Fatouh et al., 2007), butter oil (Torres et al., 2009), sheep milk fat (Spano et al., 2014) and anhydrous milk fat (Buyukbese et al., 2014). They have successfully fractionated the fats at pressures ranging from 8.9 MPa to 48.3 MPa and temperatures range from 40 ºC to 80 ºC resulting in different fractions which are useful for confectionery applications.

To date, the study on the fractionation of CNO with SFE is lacking. The aims of the present study were therefore to produce CBS from fractional extraction of CNO by SFE with CO_2, and to determine the effects of CBS in the chocolate production. The present study includes two specific objectives which were:

i. To determine the effects of different pressures and temperatures on the extraction of CNO and CNO fractions by SFE with CO_2

ii. To determine the effects of different levels of CNO fractions substituted in milk chocolate

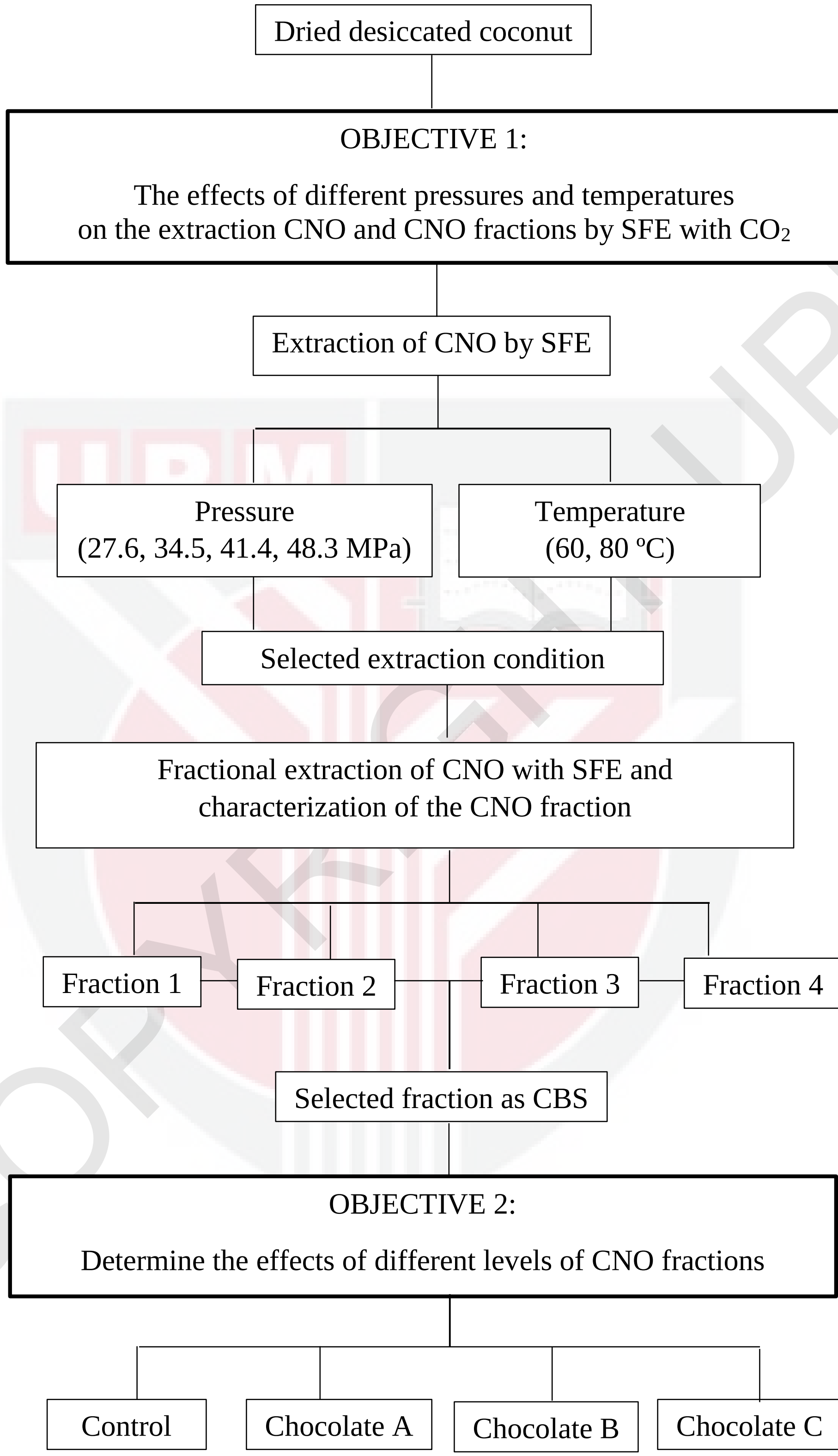

Figure 1.1: Scope of studies

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