



***EFFECTS OF DIFFERENT SOLVENT AND EXTRACTION METHODS ON
PHYTOSTEROLS AND ANTIOXIDANT ACTIVITY OF COCOA BUTTER
AND APPLICATION OF COCOA BUTTER IN SALAD DRESSING***

ROIAINI BINTI MOHAMAD

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By

ROIAINI BINTI MOHAMAD

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Master of Science**

December 2015

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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Chairman : Norhayati Hussain, PhD
Faculty : Food and Science Technology

The addition of phytosterols and antioxidant into food product may give additional health benefit towards consumers. Their stability in a food product may also decrease as the storage increase. Therefore, the objectives of this study were: i) to screen the most suitable solvent for extraction of CB using Soxhlet extraction and ii) to optimize the most suitable extraction methods in producing high amounts of phytosterols and antioxidant activity, iii) to formulate CB based salad dressing (CBSD) and iv) to determine the stability of phytosterols, antioxidant activity and physicochemical properties of CBSD during storage.

Dried cocoa beans was treated with four different types of solvents: Hexane (HE), Petroleum ether (PE), Isopropanol (PR) and Ethanol (ET) using Soxhlet extraction. Then, the selected solvent was applied in four different extraction methods: Soxhlet extraction (SE), Ultrasonic extraction (USE), Supercritical extraction CO₂ (SCO₂) and Supercritical extraction CO₂ with Ethanol (SCO₂-Ethanol). The CB extracted from selected extraction method was applied in salad dressing formulation with different ratios of cocoa butter (CB) and soybean oil (SB). The selected ratio of CB and SB (30:70) was studied for their stability during 28 days storage at two different temperatures.

The yield of CB produced from ET (14.25%) was significantly the lowest than HE and PE (43.24% and 39.31% respectively). ET had significantly ($p < 0.05$) the highest phytosterols (4974 µg/100 g of extract), DPPH assay (64.8%) and Total Phenolic Content (TPC) (22.38 mg GAE/100 g of extract). CB extracted from SCO₂-Ethanol showed significantly ($p < 0.05$) the highest content of yield (37.05%), total phytosterols (6441 µg/100 g of extract) and DPPH assay (84%) compared to SE, SCO₂ and USE method. For the physicochemical properties of CBSD, the commercial and control salad dressing (without CB) shared insignificantly ($p > 0.05$) similar characteristics (shear stress to shear rate, oscillating sweep stress and emulsion stability test) with CBSD containing 30% CB compared to other ratios.

Thus, 30% CBSD with the most stable emulsion was selected for storage study at 4 and 52°C. The physicochemical characteristic, the amount of phytosterols, DPPH assay and TPC of 30% CBSD during storage were decreased with time (from 0 day to 28 days) and increased with temperature suspected due to oxidation of the oil. Thus, the excellent stability of 30% CBSD at different temperatures for 28 days offers high potential application in food industries to produce CB based salad dressing enriched with phytosterols and antioxidants. Therefore, the selection of the solvent and the extraction method of cocoa beans is very crucial and may be applicable for the cocoa industry to broaden the application of CB as emulsion products.

Keywords: Cocoa butter, extraction method, phytosterols, antioxidant activity, salad dressing

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

**KESAN PELARUT DAN KAEDAH PENGESTRAKAN YANG BERBEZA
TERHADAP FITOSTEROLS DAN AKTIVITI ANTIOKSIDAN OLEH LEMAK
KOKO DAN APLIKASI LEMAK KOKO DALAM SOS SALAD**

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Penambahan fitosterols dan antioksidan ke dalam produk makanan boleh memberikan beberapa manfaat kesihatan tambahan terhadap pengguna. Kestabilannya di dalam produk makanan juga boleh menurun semasa simpanan. Oleh itu, objektif kajian ini adalah untuk: i) menyaring pelarut yang paling sesuai menggunakan pengekstrakan Soxhlet untuk mengeluarkan CB dan ii) mengoptimumkan kaedah pengekstrakan yang paling sesuai dalam menghasilkan jumlah yang tinggi fitosterols dan aktiviti antioksidan, iii) untuk memformulasikan sos salad berasaskan lemak koko (CBSD) dan iv) untuk menentukan kestabilan fitosterols, aktiviti antioksidan dan fizikokimia CBSD semasa penyimpanan.

Biji koko kering telah diuji menggunakan empat jenis pelarut: Heksana (HE), Petroleum eter (PE), Isopropanol (PR) dan etanol (ET) menggunakan pengekstrakan Soxhlet. Kemudian, pelarut terpilih telah diaplikasi ke dalam kaedah pengekstrakan yg berbeza: Pengekstrakan Soxhlet (SE), pengekstrakan ultrasonik (USE), Supercritical pengekstrakan CO₂ (SCO₂) dan Supercritical pengekstrakan CO₂ dengan Etanol (SCO₂-Etanol). Lemak koko yang diekstrak daripada kaedah pengekstrakan yang terpilih diaplikasikan ke dalam formulasi sos salad dengan nisbah yang berbeza lemak koko dan minyak kacang soya. Nisbah lemak koko dan minyak soya (30:70) telah dikaji kestabilannya selama 28 hari penyimpanan pada dua suhu yang berbeza.

Hasil lemak koko daripada ET (14.25%) adalah terendah berbanding HE dan PE (masing-masing 43.24% dan 39.31%). ET menghasilkan kandungan fitosterols (4974 µg/100 g ekstrak), DPPH assay (64.8%) dan jumlah kandungan fenolik (TPC) (22.38 mg GAE/100 g ekstrak) yang signifikan ($p < 0.05$) tertinggi. Lemak koko yang diekstrak daripada SCO₂-Etanol menunjukkan hasil ekstrak (37.05%), jumlah fitosterols (6441 µg/100 g ekstrak) dan DPPH assay (84%) yang signifikan tertinggi ($p < 0.05$) berbanding SE, SCO₂ dan USE. Sifat fizikokimia 30% CBSD, sos salad komersial dan kawalan (tanpa CB) menunjukkan ciri-ciri (tegasan ricih ke ricih kadar, tekanan menyapu berayun dan kestabilan ujian emulsi) tidak signifikan ($p > 0.05$) berbanding nisbah lain.

Oleh itu, 30% CBSD telah dipilih kerana menunjukkan sifat emulsi yang paling stabil dan sesuai untuk kajian penyimpanan pada 4 dan 30°C. Walau bagaimanapun, ciri-ciri fizikokimia, jumlah fitosterols, DPPH assay dan TPC 30% CBSD menurun mengikut masa (daripada 0 hari hingga 28 hari) dan meningkat mengikut suhu semasa penyimpanan disebabkan oleh pengoksidaan minyak. Oleh itu, suhu penyimpanan yang paling sesuai untuk sos salad adalah pada 4°C berbanding 52°C. Kestabilan yang sangat baik oleh 30% CBSD pada suhu yang berbeza selama 28 hari menawarkan aplikasi yang berpotensi tinggi di dalam industri makanan untuk menghasilkan sos salad berasaskan lemak koko diperkaya dengan fitosterols dan antioksidan. Oleh itu, pemilihan pelarut dan kaedah pengekstrakan biji koko adalah sangat penting dan boleh digunakan untuk industri koko bagi meluaskan penggunaan lemak koko sebagai produk emulsi.

Kata kunci: Lemak koko, kaedah pengekstrakan, fitosterols, aktiviti antioksidan, sos salad

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I certify that a Thesis Examination Committee has met on 17 December 2015 to conduct the final examination of Roiaini binti Mohamad on her thesis entitled "Effects of Different Solvent and Extraction Methods on Phytosterols and Antioxidant Activity of Cocoa Butter and Application of Cocoa Butter in Salad Dressing" 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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LIST OF ABBREVIATIONS

LD	Low density lipoprotein
HDL	High density lipoprotein
CBSD	Cocoa butter salad dressing
GAE	Gallic acid equivalents
ECE	Epicatechin equivalents
CHD	Coronary heart disease
SCO ₂	Supercritical carbon dioxide
HPLC	High Performance Liquid Chromatography
SEM	Scanning Electron Micrographs
MPa	milli Pascals,
O/W	Oil in water
W/O	Water in oil
ET	Ethanol
HE	Hexane
PE	Petroleum ether
PR	Isopropanol
DPPH	2,2-diphenyl-1-picrylhydrazyl
TPC	Total phenolic content
PV	Peroxide value
IV	Iodine value
AV	Acid value
FFS	Free fatty acid
KOH	Potassium hydroxide

USE	Ultrasonic extraction
SE	Soxhlet extraction
BPR	Back Pressure Regulator
SLI	Italian salad dresssing
PS	Phytosterols esters
UV	Ultraviolet
SBO	Soybean oil
PKDG	Palm kernel diacylglycerol
GGM	Galactoglucomannan
RME	Rapeseed oil methyl esters
FAME	Fatty acids methyl esters
FA	Fatty acid
HPKO	Hydrogenated palm kernel oil
POPs	Phytosterol oxidation products

CHAPTER 1

INTRODUCTION

The predominant raw material used for the preparation of chocolate products is cocoa beans (*Theobroma cacao* L.). Cocoa butter (CB) is a product of cocoa beans. CB handles the melting properties of chocolate and it is obtained from cocoa beans around 50±57% yield of dry weight (Steinburg et al., 2003). In order to extract CB or other fats and oils from oil contained sources, hexane as a solvent extraction has been widely used. Despite that, there is an issue on the health and safety hazards related to the use of organic solvents. Contaminants produced from CB using a hydraulic method must be removed after the process of extraction. Thus, over disposal of the toxic organic solvents and their effect on the environment has led towards safe extraction method (Nair, 2010).

At present, a number of methods engage in the extraction of CB either from mass, liquor, or from other sources, including hydraulic press, mechanical press, screw presses, supercritical extraction, and solvent extraction method (Asep et al., 2008). The hydraulic press, mechanical press, screw presses, and solvent extraction methods are not very good in extraction because (a) need high temperature, thus affects the nutritional quality of the CB, (b) unsuitable for heat sensitive labile natural compounds, and (c) contain toxic solvent in the final products which may have an effect towards human health (Hultin, 1994). The different extraction methods may produce a different yield of bioactive compounds. A study from Beveridge et al. (2005) found that supercritical CO₂ produces higher total phytosterols content compared to the traditional method using petroleum ether for grape seed oils.

Phytosterols (200±300 mg/100 g fat) of cocoa bean exist in free and esterified forms. Most abundantly found are β -sitosterol and stigmasterol that amount to 59 and 22% of total phytosterols of cocoa beans, respectively (Staphylakis and Gegiou, 1985). A natural compound such as phytosterols, tocopherol and antioxidant content shows increasing attention due to their capabilities towards health benefits today. The chemical composition of phytosterols is similar to cholesterol. However, they are only available in humans through consuming food from plant such as vegetable oils, nuts, seeds, cereals, legumes, fruits, and vegetables or industrial supplements from plant origin (Piironen et al., 2000). Nuts and oils contain higher levels (>1%) of phytosterols than fruits and vegetables (<0.05%) (Abidi, 2001).

The diet containing high amount of phytosterols and phytosterols are able to lessen the level of LDL (low density lipoprotein) cholesterol in blood serum by restraining its absorption. This helps in prevention of hypercholesterolemia and cardiovascular system diseases (Fernandes and Cabral, 2007). Phytosterols are able to enhance the function of the urinary system and eradicate the symptoms of prostate gland enlargement (Tapiero et al., 2003). In addition, phytosterols reveal the protective effect on the oxidation of lipids due to the synergistic interactions with tocopherols, and also

during high temperature processing (Polagruto et al., 2006). In most previous studies, phytosterols were added into high-fat foods, such as dressings, margarine, or spreads to facilitate their solubility. A daily intake of phytosterols in the range of 1-2 g/day is recommended for hypercholesterolemic patients (NCEP, 2001).

The suitability of solvent and extraction methods in extracting phytosterols and antioxidant content in cocoa beans is crucial for the food industry to maintain the bioactive compounds added into foods. Therefore, the application of CB with the high amount of phytosterols and antioxidant activity in emulsion product may give some additional health benefit towards consumers such as decrease the level of cholesterol in the blood, reduce heart disease and delay some types of cell damage. The addition of CB in newly formulated salad dressing may also produce different perspective for the food industry on the use of CB containing preserved bioactive compound. Therefore, the specific objectives of this study were:

- i) to screen the most suitable solvent using Soxhlet extraction in producing high amounts of phytosterols and antioxidant activity.
- ii) to optimize the most suitable extraction methods in producing high amounts of phytosterols and antioxidant activity.
- iii) to formulate cocoa butter based salad dressing (CBSD).
- iv) to determine the stability of phytosterols, antioxidant activity and physicochemical properties of CBSD during storage.

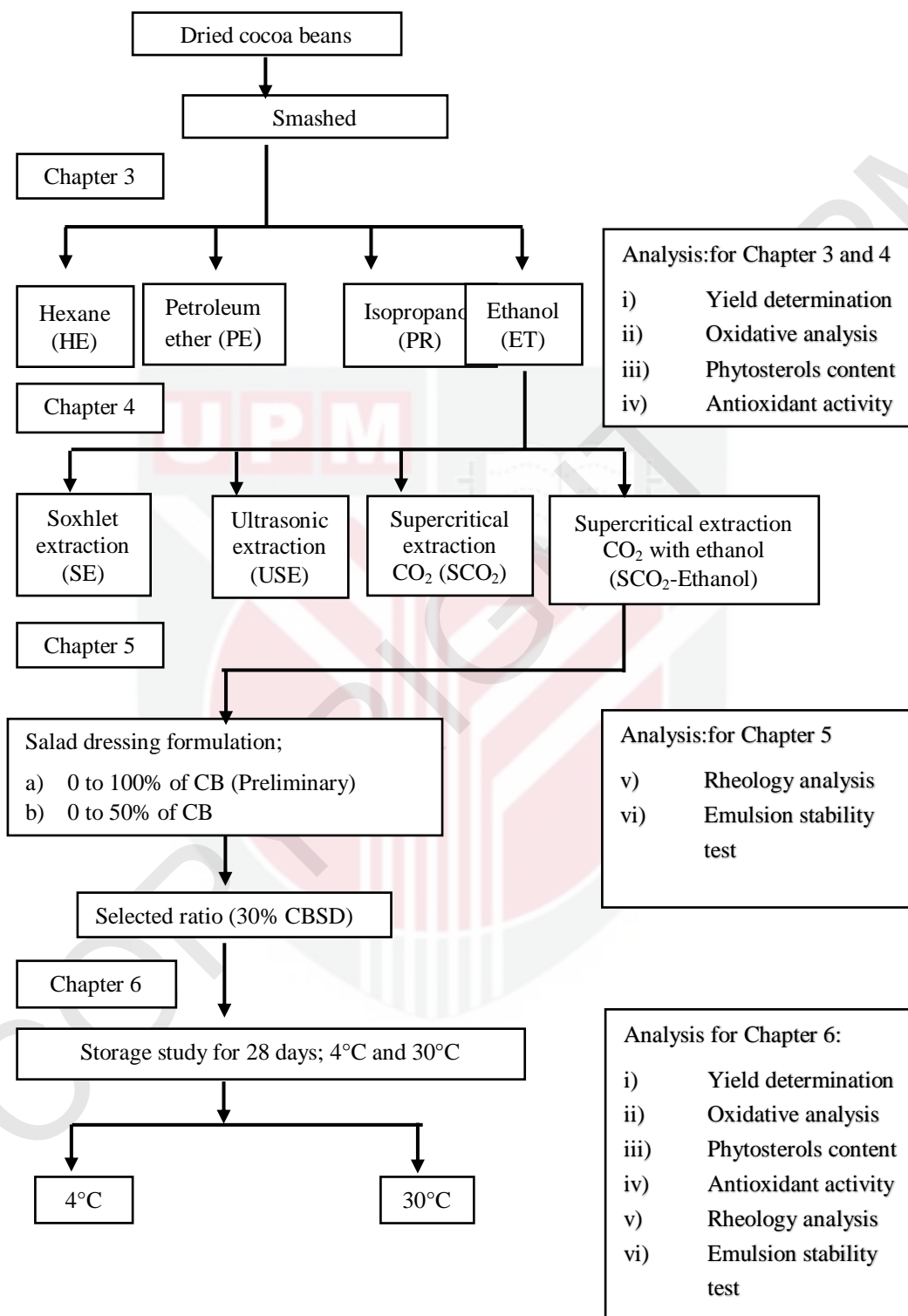


Figure 1.1. Experimental flow of the study

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