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CHARACTERISATION OF RED PALM OIL MICROCAPSULE WITH SOLUTION-ENHANCED DISPERSION BY SUPERCRITICAL CARBON DIOXIDE TECHNOLOGY

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By

LEE WAN JUN

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Chairman: Associate Professor Chong Gun Hean, PhD
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The carotenes and vitamin E (vE) in red palm oil (RPO) are highly susceptible to degradation which limits the utilisation of RPO as functional ingredient. The objective of this research was to enhance the stability of RPO and to slow down the degradation of bioactive compounds by microencapsulation using the solution-enhanced dispersion by supercritical carbon dioxide (SEDS) technology without the aid of organic solvents. This study began with the determination of RPO solubility and the concentration of carotenes, tocopherols and tocotrienols from the solubilised RPO in supercritical carbon dioxide ($scCO_2$) using the dynamic flow-through method, at different pressures (8.5– 25.0 MPa) and temperatures (40– 60°C), at a pre-determined $scCO_2$ flow rate of 2.9 g/min. The RPO had solubility of 0.5– 11.3 mg/g CO_2 and was significantly affected by pressure and temperature. The *β*-carotene recorded higher concentration in $scCO_2$ (3 x 10^{-3} μmol/mol to 17 x 10^{-3} μmol/mol) compared to *α*-carotene (2 x 10^{-3} μmol/mol to 15 x 10^{-3} μmol/mol); and tocotrienols were more soluble (0.4 μmol/mol to 26 μmol/mol) than tocopherols (5 x 10^{-2} μmol/mol to 3 μmol/mol) following the preference of γ-T_3 > δ-T_3 > α-T_3 > α-T> β-T > γ-T > δ-T. Adachi-Lu model was the best fitting model for the solubility of RPO and the concentration of bioactive compounds in $scCO_2$. The RPO was then microencapsulated with SEDS at 10.0– 15.0 MPa, 40– 60°C, and feed injection flow rate 2.5 mL/min. The microcapsules were characterised in terms of their physical, structural, and thermal properties and also their oxidative stability. Microcapsules with microencapsulation efficiencies (ME) of oil (64– 92%), retention efficiencies (RE) of carotenes (50– 82%), RE of vE (64– 94%), particle size (4– 9 μm) were obtained. Comparing to the SEDS microcapsules (SEDS-M), the spray dried microcapsules (SD-M) as the control had wrinkled surfaces with lower ME (79%), similar RE (carotene= 85%, vE= 93%), larger particle size (17 μm) and larger particle size distribution. The fatty acid composition, chemical structures and thermal properties of the oil were not altered by the SEDS process. The thermal stability, oxidative stability and retention of carotenes and vE of RPO were improved after SEDS encapsulation. Colour change and degradation in carotenes and vE concentration for microcapsules and bulk oil at different storage temperatures (25°C, 45°C, 65°C, and 85°C) for up to 28 weeks was investigated and the degradation

kinetics using rate law and Arrhenius equation were studied. The degradation of carotenes and vE was well fitted into the first-order kinetic model. A negative correlation was found with the change in colour parameters to the degradation of carotenes, and two equations to correlate the relationship were established, recording R^2 of 0.8152 for SEDS-M and 0.8283 for SD-M. In summary, the SEDS process was able to produce RPO microcapsules containing high concentration of carotenes and vE, high oil encapsulation efficiency whilst able to provide protection against the deterioration of the bioactive components and oil quality.

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

PENCIRIAN MIKROKAPSUL MINYAK SAWIT MERAH DENGAN TEKNOLOGI PERTINGKATAN PENYEBARAN LARUTAN DENGAN KARBON DIOKSIDA SUPERKRITIKAL

Oleh

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Karoten dan vitamin E (vE) dalam minyak sawit merah (RPO) sangat mudah terdedah kepada kemerosotan yang menghalang penggunaan RPO sebagai bahan berfungsi. Objektif penyelidikan ini adalah untuk meningkatkan kestabilan RPO dan melambatkan kemerosotan komponen bioaktif dengan kaedah pengkapsulan menggunakan penyebaran larutan dengan teknologi superkritikal karbon dioksida (SEDS) tanpa bantuan pelarut organik. Kajian ini bermula dengan penentuan kelarutan RPO dan kepekatan karoten, tocopherols dan tocotrienols dari RPO yang terlarut dalam karbon dioksida superkritikal ($scCO_2$) menggunakan kaedah aliran dinamik, pada tekanan (8.5– 25.0 MPa) dan suhu (40– 60°C), pada kadar arus $scCO_2$ 2.9 g/min. RPO mempunyai kelarutan 0.5–11.3 mg/g CO_2 dan terjejas dengan ketara oleh tekanan dan suhu. β-karoten menghasilkan kepekatan yang lebih tinggi dalam $scCO_2$ (3 x 10^{-3} μmol/mol hingga 17 x 10^{-3} μmol/mol) berbanding dengan α-karoten (2 x 10^{-3} μmol/mol hingga 15 x 10^{-3} μmol/mol); dan tocotrienols mempunyai larut lebih tinggi (0.4 μmol/mol hingga 26 μmol/mol) daripada tocopherols (5 x 10^{-2} μmol/mol hingga 3 μmol/mol) mengikuti keutamaan γ-T_3 > δ-T_3 > α-T_3 > α-T> β-T > γ-T > δ-T. Model Adachi-Lu adalah model terbaik untuk kelarutan RPO dan kepekatan komponen bioaktif dalam $scCO_2$. RPO itu kemudiannya dikapsulkan dengan SEDS pada 10.0–15.0 MPa, 40–60°C, dan kadar aliran sampel pada 2.5 mL/min. Mikrokapsul dicirikan dari segi sifat fizikal, struktur, dan terma dan juga kestabilan oksidatifnya. Mikrokapsul dengan kecekapan mikroencapsulasi (ME) minyak (64– 92%), kecekapan pengekalan (RE) karotene (50– 82%), RE vE (64–94%), saiz zarah (4–9 μm) telah diperolehi. Berbanding dengan mikrokapsul SEDS (SEDS-M), mikrokapsul kering semburan (SD-M) sebagai sampel kawalan mempunyai permukaan yang berkedut, dengan ME yang lebih rendah (79%), RE (karotena= 85%, vE= 93%), saiz (17 μm) dan taburan saiz zarah yang besar. Komposisi asid lemak, struktur kimia dan sifat termal minyak tidak diubah oleh proses SEDS. Kestabilan haba, kestabilan oksidatif dan pengekalan karoten dan vE RPO telah diperbaiki selepas pengkapsulan SEDS. Perubahan warna dan degradasi dalam karoten dan kepekatan vE untuk mikrokapsul dan minyak pada suhu penyimpanan yang berlainan (25°C, 45°C, 65°C, dan 85°C) selama 28 minggu serta kinetik degradasi dikaji menggunakan persamaan Arrhenius. Degradasi karoten

dan vE telah dikaitkan dengan baik dengan model kinetik pertama. Satu korelasi negatif didapati dengan perubahan parameter warna dengan kemerosotan karoten, dan dua persamaan untuk mengaitkan hubungan telah ditubuhkan, merakamkan R^2 0.8152 untuk SEDS-M dan 0.8283 untuk SD-M. Secara ringkasnya, proses SEDS dapat menghasilkan mikrokapsul RPO yang mengandungi kepekatan karoten dan vE yang tinggi, kecekapan enkapsulasi minyak tinggi dan dapat memberikan perlindungan terhadap kemerosotan komponen bioaktif dan kualiti minyak.

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

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

AARD	Average absolute relative deviation
CO_2	Carbon dioxide
C	Concentration per amount of CO_2
C	Chroma
DTG	Derivative thermogravimetric
DE	Dextrose equivalent
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
GC-FID	Gas chromatography-flame ionization detector
HPLC	High performance liquid chromatography
MC	Moisture content
MD	Maltodextrin
ME	Microencapsulation efficiency
MUFA	Monounsaturated fatty acid
NaCas	Sodium caseinate
o/w	Oil-in-water
PKO	Palm kernel oil
PO	Palm oil
PPO	Palm-pressed mesocarp fibre oil
PS	Particle size
PSD	Particle size distribution
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
RE	Retention efficiency
RPO	Red palm oil
$scCO_2$	Supercritical carbon dioxide
SD	Spray drying
SD-M	Spray dried microcapsules
SEDS	Solution-enhanced dispersion by supercritical carbon dioxide

SEDS-M	Microcapsules produced from the SEDS process
SFA	Saturated fatty acid
TGA	Thermogravimetric analysis
Totox	Total oxidation value
UFA	Unsaturated fatty acid
UV	Ultraviolet
vE	Vitamin E
p-AV	*p*-anisidine value
ρB	Bulk density
ρT	Tapped density
y_2	Solubility

CHAPTER 1

INTRODUCTION

1.1 Background

Palm oil no longer remains anonymous to the people around the globe as it is widely used in the food industry as well as in the oleochemical industry. Malaysia is currently the second largest palm oil producer and exporter, accounting for approximately 30% and 37%, respectively, with recorded export revenue of RM 64.58 billion as of year 2016 (Malaysian Palm Oil Board, 2017). Red palm oil (RPO) is well known for its high concentration of bioactive components such as carotenes and vitamin E (vE). These bioactive components are reported to provide beneficial health effects because of their antioxidant properties (Amorim-Carrilho et al., 2014; Seppanen et al., 2010).

One of the problem that RPO faces is the susceptibility of the bioactive compounds such as carotenes and vE towards degradation when exposed to the environment, especially to heat (Cardoso et al., 2015; Zeb and Murkovic, 2013). The sensitivity of carotenes and vE had limited the application of RPO as functional ingredients. Even at temperature below 100°C, the degradation of carotenes and vitamin E is evident. The exposure of carotenes to heat at 37°C for 72 hours caused 64– 68% of β-carotene and 36– 50% of α-carotene to degrade whereas at 60°C, 90– 95% of β-carotene and 85– 90% of α-carotene were loss (Anguelova and Warthesen, 2000). As for the degradation of vE, Sabliov et al. (2009) reported the degradation of α-tocopherol at temperature as low as 40°C and Nissiotis and Tasioula-Margari (2002) reported a complete degradation of α-tocopherol in virgin olive oil stored at 60°C for 30 days. The deterioration of these bioactive components not only cause the nutritional value in the RPO to be lowered, but also bring about quality changes such as lipid peroxidation, discolouration and off flavours (Soukoulis and Bohn, 2015). These issues have created limitations to the wide applications of RPO and this setback could be solved by protecting the RPO with a layer of solid matrix using the microencapsulation technology.

Different encapsulation techniques have been explored by researchers to microencapsulate different types of oil. For instance, spray drying (Binsi et al., 2017; Tolve et al., 2017), freeze drying (Fioramonti et al., 2017; Gonzalez et al., 2016), co-extrusion (Chew and Nyam, 2016), complex coacervation (Timilsena et al., 2016) and fluidised-bed agglomeration (Fuchs et al., 2006). Several methods have been used to microencapsulate palm oil, such as spray drying (Ferreira et al., 2016; Kelly et al., 2014) and complex coacervation (Rutz et al., 2017). However, there are certain drawbacks with the aforementioned encapsulation technologies. The spray-dried microcapsules are reported to have wide particle size distribution and have wrinkled surface morphology (Domian et al., 2017; Umesha et al., 2013). Besides, the high operating temperature needed for drying could potentially deteriorate the bioactive components and also, the presence of oxygen could lead to higher oxidation in oil (Ferreira et al., 2016; Heinzelmann et al., 2000). As for the complex coacervation microencapsulation, it is difficult to optimise the process because there are numerous factors and parameters to be considered (Siow, 2012). Microencapsulation with supercritical fluid (SCF) technology, the solution-enhanced dispersion by supercritical carbon dioxide (SEDS) technology is therefore introduced as an alternative to overcome the shortcomings of the aforementioned technologies. The SEDS particle formation could be carried out at mild temperatures, attributed to the low critical pressure and low critical temperature of the carbon dioxide. Hence, the degradation of thermolabile components could be potentially lowered (Nunes and Duarte, 2011). Besides, the process parameters such as pressure, temperature, feed and supercritical carbon dioxde ($scCO_2$) flow rate could be easily adjusted to produce microcapsules with desired properties (Chen et al., 2012; Yan et al., 2015; Zhao et al., 2015). Particle formation or encapsulation of different food bioactive components has been carried out using the SEDS technology (Aguiar et al., 2016; Machado et al., 2014; Zhao et al., 2015), however, the encapsulation of edible oil using the SEDS technology is lacking. Also, the reported SEDS process required the use of organic solvent which is compatible with food products (ethanol) and or incompatible (dimethyl sulfoxide, dichloromethane and acetone) to aid in the encapsulation process. The organic solvents were needed to increase the solubility of the solute (Chemat et al., 2017) with their traits of having high solubility and high expansion rate in the $scCO_2$. The use of water as the sole solvent for SEDS process has not been reported.

Hence, this work contributes by introducing the use of SEDS as an alternating technique to microencapsulate RPO without the aid from organic solvents. The SEDS microencapsulation technology is proposed to produce microcapsules with comparable or even better characteristics compared to the conventional technique (spray drying).

1.2 Objective

The main objective of this research was to enhance the oxidative stability of RPO and to slow down the degradation of bioactive components in RPO by microencapsulation using the SEDS technology without the aid of organic solvents. Emphasis was placed on studying the physical, chemical, thermal and storage properties of the RPO microcapsules produced.

The specific objectives of this study are:

i. To determine the solubility of RPO in supercritical carbon dioxide and the concentration of carotenes (α-, and β-carotene) and vE (tocopherols and tocotrienols) in the solubilised RPO per amount of carbon dioxide and to correlate the solubility data with mathematical models.
ii. To investigate the effect of SEDS operating pressure and temperature on the characteristics of RPO microcapsules.
iii. To determine the chemical structure, thermal properties, thermal stability and oxidative stability of RPO microcapsules produced from SEDS.
iv. To study degradation kinetics of carotenes and vitamin E and colour change in RPO, SEDS-M and SD-M.

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