



UNIVERSITI PUTRA MALAYSIA

**PREPARATION AND CHARACTERIZATION OF PALM-BASED
FUNCTIONAL LIPID NANODISPERSIONS**

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**PREPARATION AND CHARACTERIZATION OF PALM-BASED
FUNCTIONAL LIPID NANODISPERSIONS**

By

CHEONG JEAN NE

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

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Chairman : Tan Chin Ping, PhD

Faculty : Food Science and Technology

Poor solubility of functional lipids has made their use problematic in food industry especially in food formulations. The difficulties to find a suitable formulation or solution are even greater especially when the functional lipids are poorly soluble in both aqueous and organic solutions, which may prone to reduce bioavailability. The main objective of this study was to prepare and characterize palm-based functional lipids nanodispersions. The observations presented in this study confirmed that the nanosized droplets formed using emulsification-evaporation is relatively simple and effective technique especially for producing nanodispersions of palm-based functional lipids (tocopherols-tocotrienols and carotenoids). Droplet size can be produced in a controlled way by adjusting the processing parameters such as pressure and cycle number accordingly. This study indicated that by increasing the energy input beyond moderate pressures (20 – 80 MPa) and cycles (1 - 3) led to “over-processing” of droplets. Results have revealed that homogenization pressures have significant ($P < 0.05$) influence on the average droplet size and droplet size

distribution (PI). On the contrary, the processing cycle had not significantly effect the average droplet size and size distribution ($P > 0.05$). Preliminary studies have shown droplet diameters in the range of 90 - 120 nm for prepared α -tocopherol nanodispersions. Meanwhile, nano-droplet resulted from nanodispersions prepared with palm-based functional lipids extended from 95 – 130 nm and 140 – 210 nm for tocopherols-tocotrienols and carotenoids, respectively. During storage duration, all prepared nanoemulsions showed good physical stability. However, the content of the prepared nanodispersions was significantly ($P < 0.05$) reduced during storage. Investigation on the effect of polyoxyethylene sorbitan esters and sodium caseinate also revealed that the average droplet size significantly ($P < 0.05$) increased with increasing chain length of fatty acid and increasing the HLB value. Among the prepared nanodispersions, the palm-based tocopherols-tocotrienols nanodispersions containing Polysorbate 20 illustrated the smallest average droplet sizes and narrowest size distribution (201.8 ± 1.4 nm; PI, 0.399 ± 0.022); while palm-based carotenoids nanodispersions containing sodium caseinate had the largest average droplet size (386.3 ± 4.0 nm; PI, 0.465 ± 0.021); thus indicating more emulsifying role induced by Polysorbate 20 compared to sodium caseinate.

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

**PENYEDIAAN DAN PENCIRIAN LIPID FUNGSI NANO-SEBARAN
BERASASKAN KELAPA SAWIT**

Oleh

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Pemelarutan lipid fungsi yang lemah adalah satu masalah besar yang dihadapi oleh industri makanan khasnya dalam proses penyediaan makanan. Kerumitan untuk mendapatkan formula yang sesuai adalah lebih mencabar apabila lipid fungsi ini melarut di dalam larutan akueus dan organik dengan kadar yang lemah. Ini secara tidak langsung mungkin akan mengurangkan kadar bio-penyerapan. Objektif utama kajian ini adalah untuk menyediakan dan mencirikan nano-sebaran lipid fungsi berasaskan kelapa sawit. Hasil kajian ini menunjukkan bahawa titisan nano yang dihasilkan berkesan dengan pengemulsian dan penyejatan adalah teknik yang mudah dan efektif terutamanya dalam penyediaan nano-sebaran berasaskan kelapa sawit; (tokoferol-tokotrienol dan karotenoid). Saiz butiran dihasilkan dengan pengawalan parameter penghomogenan seperti tekanan dan kitaran. Kajian menunjukkan bahawa dengan peningkatan parameter tekanan (20 – 80 MPa) dan kitaran (1 - 3) yang melampau, titisan nano akan mengalami '*over-processing*'. Hasil kajian telah mendapati tekanan penghomogenan mempengaruhi purata saiz butiran dan taburan

butiran (PI) secara signifikan ($P < 0.05$). Sebaliknya, kitaran proses penghomogenan memberikan kesan yang tidak signifikan ($P > 0.05$) dari segi purata saiz butiran dan taburan butiran. Kajian awal yang dijalankan menunjukkan diameter butiran dalam lingkungan 90 - 120 nm bagi nano-sebaran α -tokoferol. Manakala, nano butiran yang dihasilkan daripada lipid fungsi berasaskan kelapa sawit adalah di dalam lingkungan 95 – 130 nm dan 140 – 210 nm bagi tokoferol-tokotrienol and karotenoid. Sepanjang tempoh simpanan, kesemua nano-sebaran menunjukkan kestabilan yang baik dari segi fizikal. Walau bagaimanapun, kandungan sebatian nano-sebaran menunjukkan pengurangan yang signifikan ($P < 0.05$) sepanjang tempoh simpanan. Penyelidikan berkaitan keberkesanan sistem emulsi *polyoxyethylene sorbitan esters* dan *sodium caseinate* menunjukkan peningkatan purata saiz butiran yang signifikan ($P < 0.05$) dengan pemanjangan rantaian asid lemak dan peningkatan nilai HLB. Antara nano-sebaran yang telah disediakan, nano-sebaran yang mengandungi tokoferol-tokotrienol berasaskan kelapa sawit dengan menggunakan *Polysorbate 20* menunjukkan purata saiz butiran yang paling kecil dengan taburan titisan yang paling sempit (201.8 ± 1.4 nm; PI, 0.399 ± 0.022) berbanding dengan sebatian lain yang distabilkan oleh *sodium caseinate* yang menunjukkan purata saiz butiran dan taburan butiran yang paling besar (386.3 ± 4.0 nm; PI, 0.465 ± 0.021). Ini membuktikan peranan pengemulsi *Polysorbate 20* adalah lebih sesuai digunakan untuk menghasilkan nano-sebaran jika dibandingkan dengan *sodium caseinate*.

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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.

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LIST OF ABBREVIATIONS

US	United States
R&D	Research & Development
IFT	The Institute of Food Technologist
PGE	Polyglycerol esters of fatty acids
PGME	Propylene Glycol Monosterate
HLB	Hydrophile-Lipophile Balance
PI	Polydispersity Index
PCS	Photon Correlation Spectroscopy
LD	Laser Diffraction
$D_{4,3}$	Mean droplet diameter
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
AFM	Atomic Force Microscopy
α	Alpha
β	Beta
γ	Gamma
δ	Delta

CHAPTER 1

INTRODUCTION

1.1 Introduction

Nowadays, functional lipids with high antioxidative properties constitute one of the fastest growing segments in the food ingredient market. Functional lipids such as carotenoids, phytosterols, ω -3 fatty acids, natural antioxidants and numerous other compounds are widely used as active ingredients in various industries especially in food industry. However, the poor solubility of functional lipids has made their use problematic in food industry. Most of the functional lipids are almost insoluble in water or show very low water solubility. The difficulties to find a suitable formulation or solution are even greater due to poor solubility of the functional lipids in both aqueous and organic media. Moreover, functional lipids may be prone to reduced bioavailability because of their low water solubility. In fact, poor absorption of functional lipids results in insufficient concentration leading to poor bioavailability especially after parenteral administration or transdermal application. It has been shown that smaller droplet size would increase the saturation solubility. This is because smaller droplets size increases the surface area and the dissolution velocity (Muller *et al.*, 2001).



For these reason, great attention should emphasize to find the appropriate solutions to overcome these problems. Improvement of the solubility and bioavailability of such active ingredients play an important role in future oral formulation, especially in functional foods, nutrition, medical and pharmaceutical products. The solutions to these problems should not only increases the solubility and bioavailability of such active ingredients but also provide high stability and a longer shelf life. A promising approach is by formulating as nanodispersions.

Hence, this research is carried out to demonstrate emulsification-evaporation as a simple and effective technique in producing simple oil in water emulsion system with controlled nano-sized. Subsequently, the finding will be used to verify high-pressure homogenization as a feasible methodology in preparing complex mixture of highly purified palm-based lipids nanodispersions. Lastly, the finalized operating parameters will be employed in determining the influence on non-ionic emulsifier on the characteristic properties of palm-based functional lipids nanodispersions.

Therefore, the main objectives of this study were:

- (1) To prepare and characterize nanodispersions containing α -tocopherol based on emulsification-evaporation techniques,
- (2) To prepare and characterize the physicochemical properties of prepared nanodispersions containing palm oil-based functional lipids,
- (3) To evaluate the physicochemical stability of prepared nanodispersions containing palm oil-based functional lipids.

CHAPTER 2

LITERATURE REVIEW

2.1 Nanotechnology

The word 'nano' is derived from the Greek word which brings the meaning for dwarf (Sahoo and Labhasetwar, 2003). A nanometer is equal to a billionth of a meter (10^{-9} m). For comparison, one nanometer is about 1/80,000 nm of the diameter of a human hair, or 10 times the diameter of a hydrogen atom. Nanoscale devices are 100 to 10,000 times smaller than human cells but are similar in size to large biomolecules such as enzymes and receptors (Yih and Wei, 2005; Sheetz *et al.*, 2005).

Nanotechnology is known as new techniques for making things which promises more for less: tinier, cheaper, lighter and speedier devices with greater functionality, using fewer raw materials and consuming less energy. Nevertheless, the capability of manipulating nanosystem in the nano-sized range has yield nanotechnology as one of the most significant areas, drawing intense interest. It is widely touched that it is going to revolutionize every aspect of our lives and leads to generate the new capabilities, new creations and new markets (Bhat, 2005).

Nanotechnology is becoming one of the most potential fields of exploration in the decades to come. Researches have been done globally and increasing investments are pouring from government and from industry over the world (Wonglimpiyarat, 2005).

The convergence of basic sciences such as biology, chemistry, physics and material sciences may extend the potential application of nanotechnology. Development and refinement of knowledge about manipulation of materials has led to an emerging attention in nano-sized materials which have remarkable characteristics (Mamalis, 2007). These features may yield beneficial functional physical and biological properties.

Companies in US, Japan, Europe and several other countries are attempting to position themselves to be nanotechnology leaders (Bhat, 2005). Up to 2004, total global investment was thought to be around \$6.25 billion, but this was set to rise. The USA's 21st Century Nanotechnology Research and Development Act (CNRDA, 2003) has allocated approximately \$3.75 billion to subsidize nanotechnologies from 2005-2008. The Japanese government has doubled its nanotechnology funding to \$800 million from 2001 to 2003. In Europe, nearly \$1.25 billion was spent on nanotechnology research and development (R&D) per annum, and the UK government has allocated about \$81.9 million per year from 2003-2009 for the expansion of nanotechnology (Dowling, 2004).

Many other countries have predicted that nanotechnology would be an area for their future exploitation. Table 2.1 illustrated that many Asian countries have incorporated the nanotechnology as a nationalized plans within the perspective of the country's

strategy. Thailand and Malaysia have joined the race of opportunities by implementing national policies to support nanotechnology. The National Nanotechnology Center (Nanotech) in Thailand has been set up in cooperation with the Ministry of Information and Communication Technology to educate the researchers on nanotechnology. Attempts have been made by Malaysia to set up an undergraduates and postgraduates network for nanotechnology between universities and colleges. In Singapore, the government started a joint venture with US firms in the field of nanobiology applications for the industrialization of processes. In China, the Nano Sci-Tech Industrial Park was established to undertake exploration and expansion on nanotechnology. In Korea, USD1.56 billion was spend for the development of nanotechnology R&D in order to train engineers in the emerging fields and assisted specific nano projects including nanomaterials, electronic devices and computer memories. In Taiwan, the government has implemented a nanotechnology development strategy from 2004-2008.

Table 2.1. Researches and policies in Asian countries on nanotechnology

Country	Research policies and activities
Thailand	Research activities in the field of nanotechnology are intended to respond to scientific and technological needs of Thai government's policy. The National Nanotechnology (Nanotech) is set up with an aim to increase Thailand's competitiveness. The R&D areas focused include advanced polymer, nanocarbon, nanoglass, nanoparticles, nanocoating, nansynthesis with applications to the industries of automotive, food, energy, environment, medicine and health.
Malaysia	The Malaysian government sets aside, under the eighth Malaysian Plan. USD 8 million for research in nanotechnology and precision engineering technology. The research projects in focus are nanophysics and nanochemistry. Malaysia currently invests in high-cost laboratories to incubate and develop new technologies, in attempt to shift from a traditional manufacturing and assembly base into nano-R&D.
Singapore	Singapore's government policy in nanotechnology promotion is focused on disk storage and biological fields. In 2002, the National University of Singapore Nanoscience and Nanotechnology Initiative (NUSNNI) were established as an interdisciplinary group to accelerate nanotechnology business.
China	The Chinese policy involved 'Climbing Project on Nanometer Science' (1990-1999). China has budgeted USD 240million in less than five years from the central government and approximately USD 240-360 million from local governments for nanotechnology research. Their strengths are development of nanoprobes and manufacturing processes using nanotubes.
Korea	The Korean government formulated the 'Comprehensive Plan for Nanotechnology Development' in 2001. It has also launched a National Nanotechnology Program covering various fields whereby nanomaterials are one of the key research areas. Research projects are funded jointly by the government and the private sectors. Major funding agencies are the Ministry of Science and Technology, the Ministry of commerce, Industry, and Energy. The research program funded by the Ministry of Science and Technology are mostly basic nanotechnology while the Ministry of Commerce, Industry, and Energy supports the research program close to commercialization.
Taiwan	Taiwan launched the National S7T Priority program on Nanotechnology in Taiwan (NPNT) with a budget of USD 680 million for research in nanotechnology. The implementing mechanism of fund allocation is according to a 20 +/60/20-rule, with (1) 20% of the funding to be targeted towards nanotechnology with short-term commercial potential, particularly those help upgrade the competitiveness of the traditional industries. (2) 60% of the R&D resources to be invested in the fields that will impact future competitiveness of current Taiwan hi-tech industries. (3) 20% of the project to be concentrated on the exploratory studies for potential applications that will generate innovative and new technologies.
Japan	Nanotechnology is ranked as an important field in the Second Science and Technology Basic Plan of the Japanese government. In 2002, the Japanese government announced the promotion of the 'New Industry Development Strategy' to tie nanotechnology and material science with new industries. Japan views the development of nanotechnology as the key to restoring its economy. In addition to government sponsored R&D, large corporations-Hitachi, Sony, Toray, Mitsui have invented in nanotechnology research.

Source: Wonglimpiyarat, 2005.

However, the penetration of nanotechnology in the market is still in the initial phase, indicating not only remarkable promises but also great consequences. All of these potential applications can significantly affect our lives, health and convenience, as well as our environment. Consequently, it triggers major concerns from the public. This generates a great extent of debates, both in the scientific world and the general media. Studies have revealed that human exposure to nanotechnology can be hazardous (Bainbridge, 2002; Cobb and Macoubrie, 2004). The nanotechnology involved many forms of hazard in military, environmental contamination, terrorist misuse and dislodgment of human beings. Hence, the government, researches and scientist ought to review the implications and benefits of this technology thoroughly to establish strict guidelines leading to a reliable nanotechnology (Poole and Owens, 2003; Edwards, 2005). Contrary to what scientists tend to concern about nanotechnology, Bainbridge (2002) provided an online assessment on the public perception of nanotechnology. According to this assessment, the public are incredibly enthusiastic concerning nanotechnology. As also demonstrated by Cobb and Macaoubie (2004), the public feel hopeful about nanotechnology rather than worried although public perception of nanotechnology is still in its initial step.

2.2 Food Nanotechnology

2.2.1 General Aspect

Nanotechnology is shifting out of the realm of science fiction into our buildings, drugs, cosmetics, and even nudging into our foods, beverages, and dietary

supplements. This technology has the capability to impact many aspects of food and agricultural systems. Food safety, disease treatment delivery system, new tools for molecular and cellular biology, new materials for pathogen recognition and security of the environment are crucial linkage of nanotechnology to the science and engineering of agriculture and food systems (Weiss *et al.*, 2006).

The development of new food products traditionally comprises the application of unit operations such as heat, shear, drying and freezing processes or alteration of product composition in order to generate different textures in food stuff by varying constitutions thereby attract the customers. The next wave of food innovation will budge from macroscopic scale to nano-scale. The exploration and applications of nanoscience to the food industries vary from enhancing the security of the food supply, differentiating molecules based on structure and size, nanosensors packaging or smart delivery system. These applications allow improvement to products quality while simultaneously reducing cost and enhancing productivity (Sanguansri and Augustin, 2006).

A number of groups around the world have identified the potential application of nanoscience and nanotechnology in the food industry. In 2000, Kraft company established a NanoteK Research Consortium of 15 universities and national research laboratories to carry out the research in nanotechnology for prospective food application which include food that can be customized to individual' preference and nutritional requirement, and filters that can distinguish molecules based on shape rather than size. Nowadays, more than 20 types of food and beverage in the market