PREPARATION AND TESTING OF COTTON FABRIC FINISHED WITH MICROCAPSULES CONTAINING NATURAL EXTRACTS

JIRAPORN KETWARAPORN

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Title

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By

JIRAPORN KETWARAPORN

APPROVED BY

Advisory Committee Chairperson ..... Arnee K. Aldred
(Associate Professor Dr. Arunee Kongdee Aldred)
10 / Nov / 2015

Advisory Committee Member ..... Supaporn
(Dr. Supaporn Sangsrichan)
10 / Nov / 2015

Advisory Committee Member ..... Achara Kleawkla
(Dr. Achara Kleawkla)
10 / Nov / 2015

Advisory Committee Member ..... U. Ratanakamnuan
(Dr. Usarat Ratanakamnuan)
10 / Nov / 2015

Advisory Committee Member ..... T. Chimsook
(Assistant Professor Dr. Thitiphan Chimsook)
10 / Nov / 2015

Chairperson, Committee on Doctor of Philosophy of Science Program in Applied Chemistry ..... Tanin Tangkuaram
(Dr. Tanin Tangkuaram)
10 / Nov / 2015

CERTIFIED BY GRUADUATE SCHOOL ..... Jatuphong Varith
(Assistant Professor Dr. Jatuphong Varith)
10 / NOV / 2015

Title Preparation and Testing of Cotton Fabric Finished with Microcapsules Containing Natural Extracts

Author Miss Jiraporn Ketwaraporn

Degree of Doctor of Philosophy in Applied Chemistry

Advisory Committee Chairperson Associate Professor Dr. Arunee Kongdee Aldred

ABSTRACT

Psidium guajava Linn. (guava) leaf extract and *Sapindus rarak* DC. (soap nut) fruit pericarps were studied as the antibacterial agent for application in antibacterial finished textile. This study's objective was to prepare and test antibacterial cotton fabric finished with natural extract and microcapsules containing natural extract. Microcapsules containing natural extract were prepared by *in situ* polymerization using urea and formaldehyde as wall materials for encapsulation. The weight ratio of extracts to urea were varied into five ratios ($W_{extract}/W_{urea}$); 1/5, 2/5, 3/5, 4/5 and 5/5 to optimize the highest loading capability. The quality of microcapsules was evaluated by determining the encapsulation yield (%) and the encapsulation efficiency (%). The prepared microcapsules were analyzed using Fourier transform infrared spectrometer (FTIR), optical microscopy (OM), scanning electron microscope (SEM), particle size analyzer and thermalgravimetric analysis (TGA). FTIR spectra of microcapsules containing guava leaf extract presented the absorption bands at 1444 cm^{-1} and 1118 cm^{-1}. FTIR spectra of microcapsules containing soap nut extract presented the absorption bands at 1650 cm^{-1}, 1554 cm^{-1} and 3700-3000 cm^{-1} and were broader than microcapsules without extract. The morphology of microcapsules without the extracts was spherical particles. Microcapsules containing guava leaf extract were spherical particles, but the irregular shape particles were found by increasing the ratio of core to wall beyond 3/5. Whereas microcapsules containing soap nut extract exhibit smaller spherical particles than microcapsules without the extract. The results of TGA diagrams showed that microcapsules containing guava leaf extract (at 1/5, 3/5 and 5/5 weight ratio of extract to urea) presented weight loss at temperatures of 260, 250 and 235 °C, respectively. For the TGA results of microcapsules containing soap nut extract (at 1/5, 3/5 and 5/5 weight ratio of extract to urea), they exhibited weight loss at temperatures of 260, 250 and 275 °C, respectively.

The natural extracts and microcapsules containing extracts were applied to the cotton fabrics by printing method with a binder. The qualitative antibacterial assessment was performed using agar diffusion method and AATCC 147-2004 Standard against *Esherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*). Cotton fabrics finished with guava leaf extract, microcapsules containing guava extract, soap nut extract and microcapsules containing soap nut extract showed antibacterial activity against *S.aureus*, however they were not effective against *E.coli*. The washing durability of the finished fabrics was poor at wash durability at 1 and 5 cycle times. Lastly, the free formaldehyde amount in the fabrics finished with guava leaf extract, soap nut extract, microcapsules containing guava leaf extract and microcapsules containing soap nut extract were determined to be 0.30, 0.32, 1.51 and 0.69 ppm/g of fabric, respectively.

ชื่อเรื่อง การเตรียมและทดสอบผ้าฝ้ายที่ตกแต่งสำเร็จด้วยไมโครแคปซูลบรรจุสารสกัดธรรมชาติ

ชื่อผู้เขียน นางสาวจิราพร เกตุวราภรณ์

ชื่อปริญญา ปรัชญาดุษฎีบัณฑิต สาขาวิชาเคมีประยุกต์

ประธานกรรมการที่ปรึกษา รองศาสตราจารย์ ดร.อรุณี คงดี อัลเดรด

บทคัดย่อ

สารสกัดใบฝรั่ง (*Psidium Guajava* Linn.) และสารสกัดจากเปลือกผลมะคำดีควาย (*Sapindus rarak* DC.) ถูกศึกษาเป็นสารต้านเชื้อแบคทีเรียสำหรับการประยุกต์ในสิ่งทอตกแต่งสำเร็จต้านเชื้อแบคทีเรีย วัตถุประสงค์ของการศึกษาครั้งนี้คือ เพื่อเตรียมและทดสอบผ้าฝ้ายที่มีฤทธิ์ต้านเชื้อแบคทีเรีย ซึ่งตกแต่งสำเร็จด้วยสารสกัดธรรมชาติและไมโครแคปซูลที่บรรจุสารสกัดธรรมชาติ ไมโครแคปซูลที่บรรจุสารสกัดธรรมชาติถูกเตรียมด้วยปฏิกิริยาอินซิทูพอลิเมอไรเซชัน (*in situ* polymerization) โดยใช้ยูเรียและฟอร์มัลดีไฮด์เป็นวัสดุผนังสำหรับการห่อหุ้ม อัตราส่วนของสารสกัดต่อยูเรียถูกแปรผันเป็นห้าอัตราส่วนได้แก่ 1/5, 2/5, 3/5, 4/5 และ 5/5 เพื่อให้ได้ประสิทธิภาพที่เหมาะสมต่อการกักเก็บสูงที่สุด คุณภาพของไมโครแคปซูลถูกวิเคราะห์โดยใช้เครื่องฟูเรียทรานสฟอร์มอินฟาเรดสเปคโทรมิเตอร์ (FTIR) กล้องจุลทรรศน์แบบใช้แสง (OM) กล้องจุลทรรศน์แบบส่องกราด (SEM) เครื่องวัดขนาดอนุภาคและการวิเคราะห์เชิงความความร้อน (TGA) ฟูเรียทรานสฟอร์มอินฟาเรดสเปคตราของในไมโครแคปซูลบรรจุสารสกัดใบฝรั่งปรากฏแถบการดูดซับที่ความถี่ 1444 ต่อเซนติเมตร และ 1118 ต่อเซนติเมตร ฟูเรียทรานสฟอร์มอินฟา-เรดสเปคตราของไมโครแคปซูลบรรจุสารสกัดมะคำดีควายในไมโครแคปซูลปรากฏแถบการดูดซับที่ 1650 ต่อเซนติเมตร 1554 ต่อเซนติเมตร และ 3700-3000 ต่อเซนติเมตร ซึ่งกว้างกว่าไมโครแคปซูลที่ไม่บรรจุสารสกัด ลักษณะสัณฐานของไมโครแคปซูลที่ไม่บรรจุสารสกัดมีอนุภาคเป็นทรงกลม ไมโครแคปซูลที่บรรจุสารสกัดใบฝรั่งมีอนุภาคทรงกลม แต่จะมีรูปร่างที่ไม่แน่นอน ซึ่งถูกพบได้เมื่ออัตราส่วนของสารที่กักเก็บต่อผนังมากกว่า 3/5 ขณะที่ไมโครแคปซูลที่บรรจุสารสกัดมะคำดีควายมีรูปร่างทรงกลมที่เล็กกว่าไมโครแคปซูลที่ไม่บรรจุสารสกัด ผลของไดอะแกรมจากการวิเคราะห์เชิงความร้อนแสดงให้เห็นว่า ไมโครแคปซูลที่บรรจุสารสกัดใบฝรั่ง (ที่อัตราส่วนน้ำหนักสารสกัดต่อยูเรียที่ 1/5, 3/5 และ 5/5) แสดงอุณหภูมิที่เกิดการสูญเสียน้ำหนักที่ 260, 250 และ 235 องศาเซลเซียส ตามลำดับ สำหรับผลการวิเคราะห์เชิงความร้อนของไมโครแคปซูลที่บรรจุ

สารสกัดมะคำดีควาย (ที่อัตราส่วนน้ำหนักสารสกัดต่อยูเรียที่ 1/5, 3/5 และ 5/5) แสดงอุณหภูมิที่เกิดการสูญเสียน้ำหนักที่ 260, 250 และ 275 องศาเซลเซียส ตามลำดับ

หลังจากนั้น สารสกัดธรรมชาติและไมโครแคปซูลที่บรรจุสารสกัดได้ถูกนำมาใช้กับผ้าฝ้ายโดยวิธีการพิมพ์กับตัวเชื่อมจับ การประเมินฤทธิ์ต้านเชื้อแบคทีเรียเชิงคุณภาพทดสอบโดยใช้วิธีเอก้าร์ดิฟฟิวชัน (agar diffusion method) และวิธีมาตรฐานเอเอทีซีซีหนึ่งสี่เจ็ด-สองพันสี่ (AATCC 147-2004) ทดสอบกับเชื้อเอสเซอริเชียคอไล (*Esherichia coli*, *E.coli*) และเชื้อสเตปฟิโลคอกคัสออเรียส (*Staphylococcus aureus*, *S.aureus*) ผ้าฝ้ายที่ผ่านการตกแต่งสำเร็จด้วยสารสกัดใบฝรั่ง ไมโครแคปซูลที่บรรจุสารสกัดใบฝรั่งสารสกัดมะคำดีควาย และไมโครแคปซูลที่บรรจุสารสกัดมะคำดีควายมีฤทธิ์ต้านเชื้อเชื้อสเตปฟิโลคอกคัสออเรียส แต่ไม่มีผลต่อเชื้อแบคทีเรียเอสเซอริเชียคอไล ผ้าที่ตกแต่งสำเร็จไม่มีความคงทนต่อการซักล้างหลังจากการซักล้างครั้งที่ 1 และ 5 สุดท้ายปริมาณฟอร์มัลดีไฮด์อิสระบนผ้าที่ตกแต่งสำเร็จด้วยสารสกัดใบฝรั่ง สารสกัดมะคำดีควาย ไมโครแคปซูลที่บรรจุสารสกัดใบฝรั่ง และไมโครแคปซูลที่บรรจุสารสกัดมะคำดีควาย มีค่าเท่ากับ 0.30, 0.32, 1.51 และ 0.69 หนึ่งส่วนในล้านส่วน (ppm) ต่อน้ำหนักเป็นกรัมของผ้า ตามลำดับ

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Jiraporn Ketwaraporn

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ABBREVIATIONS

cm^{-1}	per centimeter
CFU	colony forming unit
EE	encapsulation efficiency
EY	encapsulation yield
l	liter
m	meter
MCs	microcapsules
mg/l	milligram per liter
mm	millimeter
nm	nanometer
ppm	parts per million
PUF	poly(urea-formaldehyde)
UF	urea-formaldehyde
µl	microliter
µm	micrometer

CHAPTER 1

INTRODUCTION

1.1 Problem

Increasing environmental awareness has made people realize the importance of living in a world with a clean atmosphere (Shahid et al., 2012). The population explosion and the environmental pollution in the recent years have forced researchers to find new health and hygiene related products for the wall being of mankind (Giri Dev et al., 2009). The customer is increasing in awareness and concern for the safety of products. Thus, there is a need to develop textiles that are resistant to microbes (bacteria, mould or fungi) as the textile substrates find various applications such as masks, hospital covers, and surgical gowns apart from conventional apparel usage (Ferreira et al., 2012).

Textiles find immense applications in day to day life and there has been a growing need to develop finishes for textile materials that can offer improved protection to the users from microbes, which cause numerous problems (Giri Dev et al., 2009). Because the large surface areas and ability which retain moisture make textile fabrics more prone to bacterial growth. Moreover, clothing and other textile materials can act as carriers for microorganisms such as pathogenic or odour-generating bacteria and moulds and when in contact with the human body, they offer an ideal environment for microbial growth, providing oxygen, water and warmth, as well as nutrients from spillages and body exudates (Hashem et al., 2009).

Cotton fabric is carried out in this work because cotton textile is a common problem since their porous hydrophilic structures that retain water, oxygen and nutrients which provide a perfect environment for growth of microorganisms (Abdel-Halim et al., 2011).

Some microorganisms, for example, *Staphylococcus aureus* (*S. aureus*) is a major disease causing bacteria that exist in the arm pit, inner elbow, between mid-buttocks, sides of groin and bottom of the heel. *Escherichia coli* (*E. coli*) is also considered a potential pathogen and resides mainly in the small intestine. However, they also can be found in inguinal and perineal areas contaminated by urine and feces. Because of the direct contact on fabrics with human skin, most bacteria can transfer and propagate between the two. Furthermore, bacteria can reside fabrics causing fabric discoloration, skin disease and unpleasant odors (Perera et al., 2013).

Hence, antimicrobial finishes on fabrics are developed to protect consumer against the disease or odor causing microorganisms.

Antimicrobial finishing, by definition, inhibits the growth of or kills microorganisms (bacteria and fungi, and biostats) (Abdel-Halim et al., 2011). Antimicrobial finishes can be divided into biocides. The major classes of microbial agents for textile include quaternary ammonium compounds, Nhalamines, chitosan, polybiguanides, triclosan, nanosized inorganic particles and bioactive plant based products (Ibrahim et al., 2011).

Various antimicrobial technologies have been developed to protect various materials from antimicrobial damage and to prevent microbial infection (Koh and Hong, 2014). Thus, textiles are treated with various compounds. Many commercial products are currently available in the market with a range of antimicrobial properties under different trade name for textile industry. Almost of products are made from synthetic agents, for example, organo-metallics, phenols, quaternary ammonium salts and organosilicons. Even though the synthetic antimicrobial agents showed high effective to inhibit the microbes, but they were caused of the hazards risk to human health (Khan et al., 2011). Different chemicals and heavy metal are non-biodegradability. Hence, a natural extract finished fabrics are considered to alternative material for this work.

Psidium guajava Linn., commonly known as guava, belongs to family of *Mystraecea* and is native plant in the South America, European, Africa and Asia. Guava leaves are rich in flavonoids and phenols (Venkatachalam et al., 2012, Gutiérrez et al., 2008). The extract of guava leaves was found to inhibit the growth of *Bacillus cereus*, *Escherichia coli*, *Proteus* spp., *Pseudomonas aeruginsa*, *Salmonella enteritidis*, *Shigella* spp. and *Streptococcus mutans* in vitro agar test (Gutiérrez et al., 2008). Its antimicrobial activities were beneficial application in antimicrobial finishing textile.

Sapindus rarak DC. (Sapindaceae) or soap nut is a tall tree which originated in South East Asia and now widely distributed in Asia and Africa. Its fruits have pericarps which are soft and brown color and become dark brown when it is dried. The pericarps contain a foaming agent and have been used as a natural soap (Wina et al., 2005a). Whole of *S. rarak* DC. fruit extract contained saponins. Saponins are bioactive compounds and the chemical structure of saponins is composed of glycone (saccharide side groups) and aglycone (known as sapogenin).

Saponins have several biological and pharmacological properties such as anti-inflammatory, antifungal/antiyeast, antibacterial/antimicrobial and antiparasasitic activity (Wina et al., 2005b). These activities were of interest to applications in textile to produce a functional fabric.

In recent years, a number of commercial applications of microencapsulation in textile are growing. Microencapsulation technique is widely spread technique using for developing new product. It is a process by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of a polymer (Dubey et al., 2009). This technique is used to protect active agents from the environment and to control release of the active components for long-acting release. The aim of this work was the encapsulation of guava leaf extracts and soap nut fruit pericarps extracts into urea-formaldehyde microcapsules by in situ polymerization to be developing an eco-friendly natural antimicrobial finish in textiles. Moreover, the research will study the efficiency of cotton fabrics finished with microcapsules containing the natural extracts, the assessment of antimicrobial activity and wash durability.

1.2 Research objectives

1.2.1 To extract polyphenol compounds and saponins from plant

1.2.2 To synthesis urea-formaldehyde microcapsules containing plant extracts

1.2.3 To prepare cotton fabric finished with microcapsules containing plant extracts by printing method

1.2.4 To prepare and test antibacterial cotton fabric

1.3 Scope

1.3.1 Literature survey of microencapsulation was carried out.

1.3.2 Polyphenol compounds and saponins were extracted from *Psidium Guajava* Linn. leaf and *Sapindus Rarak* fruit pericarps, respectively.

1.3.3 The extracts of *Psidium Guajava* Linn. leaf and *Sapindus rarak* fruit pericarps were determined for total phenolic content and saponins content and antibacterial activity, respectively.

1.3.4 Urea-formaldehyde microcapsules containing the extracts of *Psidium Guajava* Linn. leaves and *Sapindus rarak* fruit pericarps were prepared.

1.3.5 %Encapsulation efficiency (%EE), %encapsulation yield (%EY) and of the prepared microcapsules were determined.

1.3.6 Characterization of microcapsules by Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectrometer (FT-IR), Particle size analyzer and Thermal gravimetric analysis (TGA) were carried out.

1.3.7 Cotton fabrics were finished with urea-formaldehyde microcapsules containing the extracts.

1.3.8 Finished cotton fabrics were characterized by Scanning Electron Microscope (SEM) and Optical microscope (OM).

1.3.9 Antibacterial activity and formaldehyde amount of cotton fabrics finished with antibacterial agent were assessed.

1.3.10 Conclusions were drawn from the experimental results.

1.4 Usefulness of the research

1.4.1 Polyphenol compounds and saponins could be extracted from plant extracts.

1.4.2 Urea-formaldehyde microcapsules containing plant extracts were synthesized by *in situ* polymerization method.

1.4.3 Antibacterial textile was prepared by incorporation of urea-formaldehyde microcapsules containing plant extracts.

CHAPTER 2

LITERATURE REVIEWS

2.1 Microencapsulation

Microencapsulation technology was established in 1942 by Barett K. Green, the father of microencapsulation. He had developed a working method of microencapsulating ink and a prototype carbonless paper. Carbonless paper was composed of three layers including of the paper, a film of acid-sensitive dye packaged in microcapsules and a layer of acidic clay. The dye microcapsules were encapsulated with gelatin by coacervation. The pressure from writing broke the dye microcapsules on the underside of each sheet. After the dye was released, it reacted with acidic layer on the surface of the next sheet and it was transparent to dark blue or black (Veršic, 2013). This technique has long used for the preparation of capsules containing an active ingredient in various industries, for example pharmaceutical, agricultural, food, cosmetic and textile industries (Ghosh, 2006).

The definition of microencapsulation is preferred to a process by which very tiny droplets or particles of liquid or solid material are surrounded or encapsulated with a continuous film of a polymeric material (Nelson, 1991). The product from microencapsulation process is termed a microcapsule (Ghosh, 2006). As a consequence of the development and specialization of microencapsulation technologies and applications, microencapsulation products differ in structure and terminology are described in **Table 1**. Some microcapsules/microspheres which their diameter is in the nanometer range are referred to as nanocapsules/nanospheres to emphasize their smaller size (Dubey et al., 2009).

2.1.1 The component of microcapsule

Generally, microcapsules can be divided into two parts, the core and the shell (Ghosh, 2006). The schematic of microcapsule is shown in **Figure 1**.

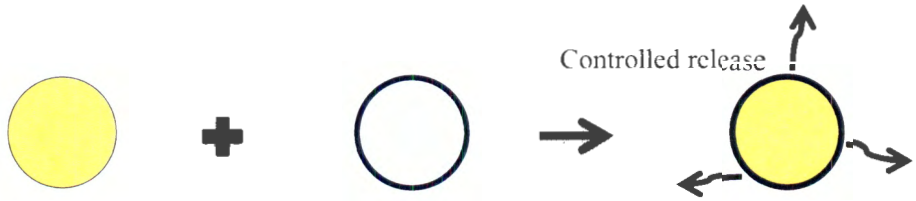
A. Core

The core material in microcapsule is referred as the specific material to be coated and may present in the term of a solid, liquid or gas. The material inside the microcapsule is also called internal phase or fill. It contains the active ingredient such as drugs, dyes, catalysts, flame

retardants and nanoparticles. Mostly the core materials are in the form of a solution, dispersion or emulsion. The composition of the core material can be varied so microcapsule presents a wide range of possible configurations (as shown in **Table 2**). The performance of microcapsule properties, flexibility and utilization may influence the configuration of the capsule (Ghosh, 2006; Bansode et al., 2010). Moreover, the core contents are released under controlled conditions to suit a specific purpose (Cheng et al., 2008). **Table 3** shows some example of core material and its characteristic in the target application for encapsulation (Umer et al., 2011).

Table 1 Terminology of microencapsulation products (Boh et al., 2008)

Terminology	Description	Size range	Schematic illustration
Microcapsules	Products from coating liquid nuclei and solid walls.	μm	
Nanocapsules	Similar to microcapsules but smaller.	nm	
Microspheres or microparticles	The core and the wall are solid and the wall contains a porous matrix.	μm	
Nanospheres or nanoparticles	Similar to microspheres but smaller.	nm	
Liposomes	The wall is lipid (unilamellar or multilamellar)	μm to nm	
Niosomes	Similar to liposomes but the wall is synthetic amphiphylic molecules.		



Core materials	Wall shell	Microcapsule
Skin moisturizing agents.	Natural polymer.	Pressure, Friction,
Skin cooling agents.	Semi-synthetic polymer.	Diffusion.
Vitamins&pro-vitamins.	Synthetic polymer.	Wall dissolution
Essential oils, etc.		Biodegradation.

Figure 1 Simple structure of a microcapsule (Cheng et al., 2008)

B. Wall

(Mishra et al., 2013; Bansole et al., 2010; Teixaira de Silval et al., 2014)

The coating material that surrounds the active ingredient is also wall, shell, coating, external phase or membrane. It is cohesive with the core material either by chemically compatible or nonreactive with the core material and can designs to provide the desired coating properties such as strength, flexibility, impermeability, optical properties and stability. The optimal wall material is very important since it affect to the encapsulation efficiency and stability of the microcapsules.

The ideal wall material should have the properties as follow;

- Not react or inert with the core
- Ability to seal and maintain the core inside the capsule
- Ability to provide maximum protection to the core against adverse conditions
- Lack an unpleasant taste in the case of food application
- Economic viability
- Controlled release under specific conditions
- Soluble in an aqueous media or solvent or melting
- Can be flexible, brittle, hard, thin etc.

However, most wall material do not have all the desired properties, a common practice involves mixing two or more materials. The wall material can be divided by the source into two groups; synthetic polymers and natural polymers.

For example of synthetic polymers are composed of

a) Non-biodegradable polymers e.g. polymethyl methacrylate (PMMA), acrolein, glycidyl methyacrylate epoxy polymers

b) Biodegradable polymers e.g. lactides, glycolides and their copolymers, polyalkyl cyanoacrylates polyanhydrides

and the example of natural polymers are composed of

a) Proteins e.g. albumin, gelatin, collagen

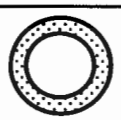
b) Carbohydrates e.g. agarose, carrageenan, chitosan, starch, gum

c) Chemically modified carbohydrates e.g. polydextran, polystarch

d) Lipids e.g. paraffin, beeswax

The characteristic of polymer may be made permeable, semi-permeable or impermeable property of wall material. Permeable wall is used to releasing application, whereas semi-permeable wall is usually impermeable to the core material but allow low molecular weight liquid throughout inside the wall. From this result, the capsule is able to absorb substances from the environment and to release them again when new substances entrance to the capsule. The impermeable wall surrounds and protects the core material form the environment so the core will release by the interference from the outside such as pressure, solvent, light and strong acid or base. The releasing rate is mainly controlled by the thickness and its pore size of the wall material (Ghosh, 2006).

Table 2 Capsule configurations (Nack, 1970)

Core	Wall	Type	Configuration
Liquid or gaseous	Solid	Spherical core	
Solid	Solid	Irregular particle	
Liquid slurry	Solid	Liquid phase suspended solids	
Spherodized solid	Solid	Solid matrix dispersed solids	
		Multiple wall capsules	

2.1.2 Reasons for microencapsulation (Umer et al., 2011)

The reasons for microencapsulation are countless. There are various reasons which involving the principle are:

- To protect the active ingredients from the environment
- To convert liquid active compounds into a dry solid system
- To separate incompatible ingredients for functional reasons
- To mask undesired properties of the active ingredients
- To protect the immediate environment of the microcapsules from the active ingredients
- To control release of the active ingredients for delayed (timed) release or long acting release

Table 3 Core material and its characteristics (Umer et al., 2011)

Core material	Characterization	Encapsulation purpose	Product
Acetaminophen	Slightly water soluble solid	Taste masking	Tablets
Aspirin	Slightly water soluble solid	Taste masking, sustained release, reduce gastric irritation and separation of incompatibles	Tablets or capsule
Isosorbide dinitrate	Water soluble solid	Sustained release	Capsule
Liquid crystals	Liquid	Conversion of liquid to solid and stabilization	Flexible film for thermal mapping of anatomy
Progesterone	Slightly water soluble solid	Sustained release	Varied
Patassium chloride	Highly water soluble solid	Reduce gastric irritation	Capsule
Urease	Water soluble enzyme	Perm selectivity of enzyme, substrate and reaction products	Dispersion
Vitamin A palmitate	Nonvalatile liquid	Stabilization of oxidation	Dry powder

2.1.3 Release mechanisms

The factors for release mechanisms are mainly caused by diffusion, degradation, solvent, pH, temperature and pressure. A combination of two or more factor is controlled in practice.

A. Diffusion

When the microcapsule wall is intact, diffusion occurs especially and the release rate is governed by the chemical properties of the core and the wall material and some physical properties of the wall. In addition, some acids can be released during a process step but protected

by another step. In other cases, some preservatives are required at the product surface, but their spread to other parts must be controlled (Azeredo, 2005)

B. Degradation

Degradation release occurs because of enzymes such as proteases degrade proteins or lipases degrade lipids (Rosen, 2006). An example is reducing the time required for the ripening of cheddar cheese by 50% compared with the conventional ripening process (Hickey et al., 2007).

C. Solvent

The wall material can dissolve completely when it is contacted with a solvent. The wall quickly released the core or start to expand, favoring release. For example, microencapsulation of coffee flavors improves the protection from light, heat and oxidation when in the dry state, but the core is released upon contact with water (Frascarell et al., 2012).

D. pH

The pH release occurs because pH changes can result in alterations in the wall material solubility, enabling the release of the core. For example, probiotic microorganisms can be microencapsulated to resist the acid pH of the stomach and only be released in the alkaline pH of the intestine (Toldrá and Reig, 2011).

E. Temperature

Changing temperature can promote core release. There are two different concepts: temperature-sensitive release, reserved for materials that expand or collapse when a critical temperature is reached, and fusion activated release, which involves melting of the wall material due to temperature increase. An example is the fat-encapsulated cheese flavor used in microwave popcorn, resulting in the uniform distribution of the flavor: the flavor is released when the temperature rises to 57-90°C (Park and Maga, 2006).

F. Pressure

Pressure release occurs when a pressure is applied to the capsule wall, such as the release of some fl avors during the mastication of chewing gum (Wong et al., 2009).

The release mechanisms of the core contents vary depending on the selection of wall materials and more importantly, its specific end uses. The core content may be released by

friction, pressure, change of temperature, diffusion through the polymer wall, dissolution of the polymer wall coating, biodegradation, etc.

Currently, microencapsulation technology is rapidly developing in the field of chemical finishing because of its versatility and flexibility. One major advantage of using microencapsulation technology is its ability to protect the active ingredients from hazardous environments such as oxidization, heat, acidity, alkalinity, moisture or evaporation. It also simultaneously, protects the ingredients from interacting with other compounds in the system, which may result in degradation or polymerization. Another important advantage of this versatile technology is its controlled release properties that seem to be the best choice for increasing efficiency and minimizing environmental damage.

2.1.4 Morphology of microcapsules (Ghosh, 2006; Cheng et al., 2008)

The morphology of microcapsules depends on the core material and the deposition process of the wall. Their morphology can be regular or irregular shapes and is classified on the basic of their morphology into three types, mononuclear, polynuclear and matrix (as shown in **Figure 2**).

First, monocored microcapsules contain a single hollow chamber inside the capsule. Second, the polycore microcapsules have a many of different sized cores within the wall. Third, the matrix type has the active ingredients integrated within the matrix of the call material in microcapsules. However, the morphology microcapsules of the internal structure depend largely on the selected wall materials and the microencapsulation methods that are employed.

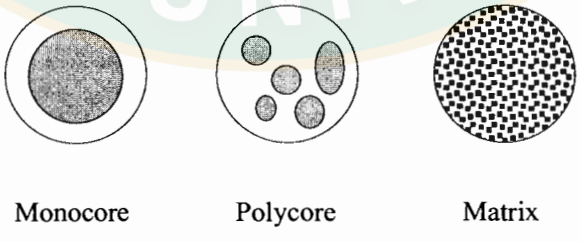


Figure 2 Morphology of microcapsules (Ghosh, 2006).

2.1.5 Microencapsulation techniques

There are numerous preparation technologies available for the encapsulation. In generally, microencapsulation techniques are categorized into two basic groups, chemical process and physical process (as shown in **Table 4**). Recently, the different microencapsulation techniques are more relevant to the coating industries and provide a comprehensive review of developed methods. Thus, theirs name is subdivided into physic-chemical process and physic-mechanical process. Various techniques of microencapsulation and some their relative particle size ranges are mentioned in **Table 5**.

Table 4 Different techniques used for microencapsulation (Ghosh, 2006)

Chemical processes	Physico-chemical processes	Physico-mechanical
Interfacial polymerization	Coacervation	Spray drying
In situ polymerization	Layer-by-layer assembly	Multiple nozzle spraying
Polycondensation	Sol-gel encapsulation	Fluid bed coating
	Supercritical CO_2 assisted microencapsulation	Centrifugal techniques
		Vacuum encapsulation
		Electrostatic encapsulation

Table 5 Microencapsulation and some their relative particle size ranges (Ghosh, 2006; Jyothi et al, 2012)

Process	Size range (μm)
Chemical processes	
- Interfacial polymerization	0.5-1000
- In situ polymerization	0.5-1000
- Polymer-polymer incompatibility	0.5-1000
Physico-chemical processes	
- Coacervation	2-1200
- Layer-by-layer assembly	0.02-10
- Sol-gel encapsulation	2-20
Physico-mechanical processes	
- Co-extrusion	250-2500
- Spray drying	5-5000
- Rotating disk	5-1500
- Fluid bed coating	20-1500

A. Interfacial polymerization (Jyothi et al., 2010; Cheng et al., 2008)

The wall material is formed on the surface of a droplet or particle by polymerization of reactive monomers. The multifunctional monomer is dissolved in the liquid core material and dispersed in aqueous phase containing dispersing agent. Monomers, multifunctional acid chlorides and multifunctional isocyanates, are usually used either individually or in combination. After that, the polymerization reaction is occurred rapidly at interface and then produced as a capsule wall. Although, the liquid and solid can be encapsulated by interfacial polymerization, but the type of reaction is different. For example, a polyuria wall will be generated when isocyanate reacts with amine, whereas polynylon or polyamide will be formed when acid chloride reacts with amine

B. *In situ* polymerization (Jyothi et al., 2010)

The polymer wall of in situ polymerization generates since the monomers are added to encapsulation reactor which is likely to interfacial polymerization. In this process, the reactive agents are not added to the core material. When the polymerization run exclusively in the continuous phase and is on the continuous phase side of the interface formed by the dispersed core material and continuous phase. Prepolymer is formed to a low molecular weight in initial step and then it deposits on the surface of the dispersed core material to form a solid capsule wall.

C. Coacervation (Ghosh, 2006)

Coacervation is the first systematic approach of phase separation that is a partial desolvation of a homogeneous polymer solution into a polymer-rich phase (coacervate) and the poor polymer phase (coacervation medium). This was realized by Bungenberg and colleagues. They were termed such a phase separation phenomenon as coacervation. This was the first reported process to be adapted for the industrial production of microcapsules. Currently, two methods for coacervation are available, simple and complex processes. The mechanism of microcapsule formation for both processes is identical, except for the way in which the phase separation is carried out. In simple coacervation a desolvation agent is added for phase separation, whereas complex coacervation involves complexation between two oppositely charged polymers.

Complex coacervation

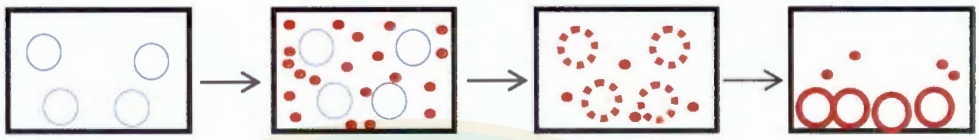
Complex coacervation is carried out by mixing two oppositely charged polymers in a solvent (usually water) and the process is shown schematically in **Figure 3.**

There are three basic steps in complex coacervation.

1. Preparation of the dispersion or emulsion. The core material (usually an oil) is dispersed into a polymer solution such as a cationic aqueous polymer.

2. Encapsulation of the core. Polymer (anionic, watersoluble) solution is then added to the prepared dispersion. The wall material is deposited onto the core occurs when the two polymers form a complex. This process is triggered by the addition of salt or by changing the pH, temperature or by dilution of the medium. The shell thickness can be obtained as desired by controlled addition of the second polymer.

3. Stabilization of the encapsulated particle. The prepared microcapsules are stabilized by crosslinking, desolvation or thermal treatment.



(a) (b) (c) (d)

Figure 3 Schematic of the coacervation (a) core material dispersion in solution of wall material (b) separation of coacervate from solution (c) coating of core material by microdroplets (d) coalescence of coacervate to form continuous wall around core (Ghosh, 2006).

D. Spray drying (Cheng et al., 2008; Ghosh, 2006)

Microencapsulation by spray drying is a low cost commercial process which is the favorite process for using encapsulation of fragrances, oils and flavors. The active material or core is dissolved or suspended in a melt or polymer solution, sprayed into a hot chamber and becomes trapped in the dried particle. **Figure 4** illustrates the process of microencapsulation by spray drying. The well material solidifies onto the core as the solvent evaporates such that the microcapsules obtained are of polynuclear or matrix type. Water soluble polymers are mainly used as shell materials because solvent-borne systems produce unpleasant odors and environmental problems. The disadvantage of this technique are some low boiling point aromatics can be lost during the drying process and the core material may also form on the surface of the capsule, which allows for oxidation and possible scent changes of the encapsulated product.

E. Centrifugal extrusion (Cheng et al., 2008; Jyothi et al., 2010)

In centrifugal extrusion processes, liquids are encapsulated by using a rotating extrusion head with concentric nozzles (**Figure 5**). The core and the wall materials should be immiscible with one another. The fluid core material is pumped through a central tube while the liquefied wall material is pumped through a surrounding annular space. The continuous wall material is formed across a circular orifice at the end of the nozzle and the core material flows into the membrane, causing the extrusion of a rod of material. Droplets break away from the rod

and are solidified by cooling or gelling bath which depend on the composition and the property of the wall material. The Solid capsules are removed by filtration or mechanical means and the immiscible carried fluid is reheated and recycled after passing through the files. Different types of extrusion nozzles have been developed in order to optimize the process.

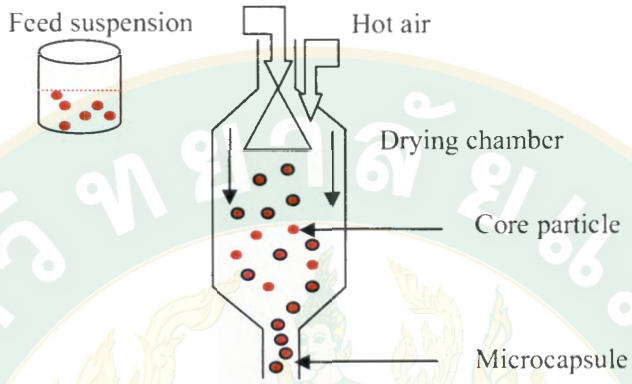


Figure 4 Schematic of spray drying (Ghosh, 2006)

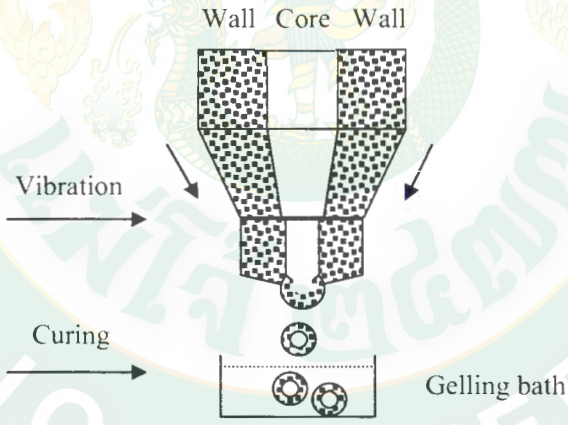


Figure 5 Schematic of centrifugal extrusion (Ghosh, 2006)

F. Fluidized-bed technology (Ghosh, 2006: Jyothi et al., 2010)

Fluid bed coating, another mechanical encapsulation method, is restricted for encapsulation of solid core materials, including liquids absorbed into porous solids with optimal heat exchange. The liquid coating is sprayed onto the particles and the rapid evaporation helps in the formation of an outer layer on the particles. The capsules are then solidified by cooling or

solvent vaporization. Different types of fluid-bed coaters include top spray, bottom spray, and tangential spray (**Figure 6**). This technique is used extensively to encapsulate pharmaceuticals.

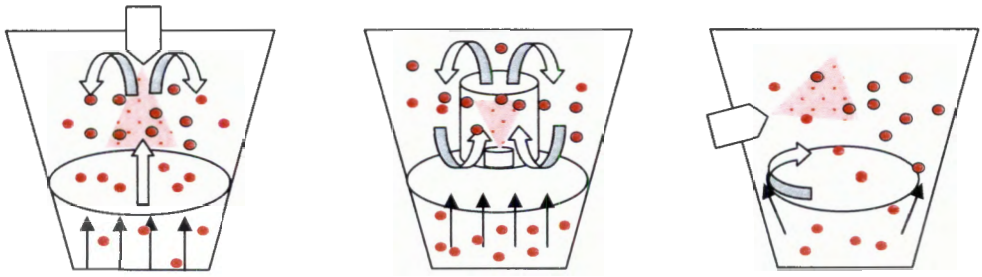
First, in the top spray system the coating material is sprayed downwards on to the fluid bed such that as the solid or porous particles move to the coating region they become encapsulated.

Second, the bottom spray is also known as "Wurster's coater". This technique uses a coating chamber that has a cylindrical nozzle and a perforated bottom plate. The cylindrical nozzle is used for spraying the coating material. As the particles move upwards through the perforated bottom plate and pass the nozzle area, they are encapsulated by the coating material. The coating material adheres to the particle surface by evaporation of the solvent or cooling of the encapsulated particle. This process is continued until the desired thickness and weight is obtained. Although it is a time consuming process, the multilayer coating procedure helps in reducing particle defects.

Last, the tangential spray consists of a rotating disc at the bottom of the coating chamber, with the same diameter as the chamber. During the process the disc is raised to create a gap between the edge of the chamber and the disc. The tangential nozzle is placed above the rotating disc through which the coating material is released. The particles move through the gap into the spraying zone and are encapsulated. As they travel a minimum distance there is a higher yield of encapsulated particles.

G. Spining disk (Ghosh, 2006)

A schematic diagram of the process is shown in **Figure 7**. Suspensions of core particles in liquid shell material are poured into a rotating disc. The core particles were coated with the shell material because of the spinning action of the disc. The shell material is solidified by external means (usually cooling). Then, the coated particles and the excess shell material are then cast from the edge of the disc by centrifugal force. This technology is rapid, cost-effective, simple and has high production efficiencies. For optimum encapsulation, spherical core particles with diameters of ~100 to 150 μm and rapidly cooling shell materials are required.



(a) (b) (c)

Figure 6 Schematic of fluidized-bed technology (a) top spray (b) bottom spray and (c) tangential spray (Ghosh, 2006)

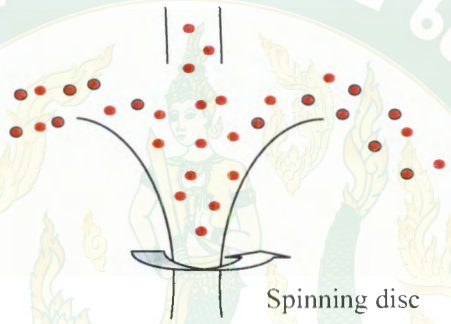


Figure 7 Schematic of spining disk (Ghosh, 2006)

Although several of alternative microencapsulation techniques are available but no single method is suitable for encapsulating different types of core material. Commonly, the preparation of microcapsules should be concern of satisfy certain criteria, like basic understanding of the general properties of microcapsules, such as the nature of the core and coating materials, the stability and release characteristics of the coated materials and the microencapsulating methods (**Figure 8**).

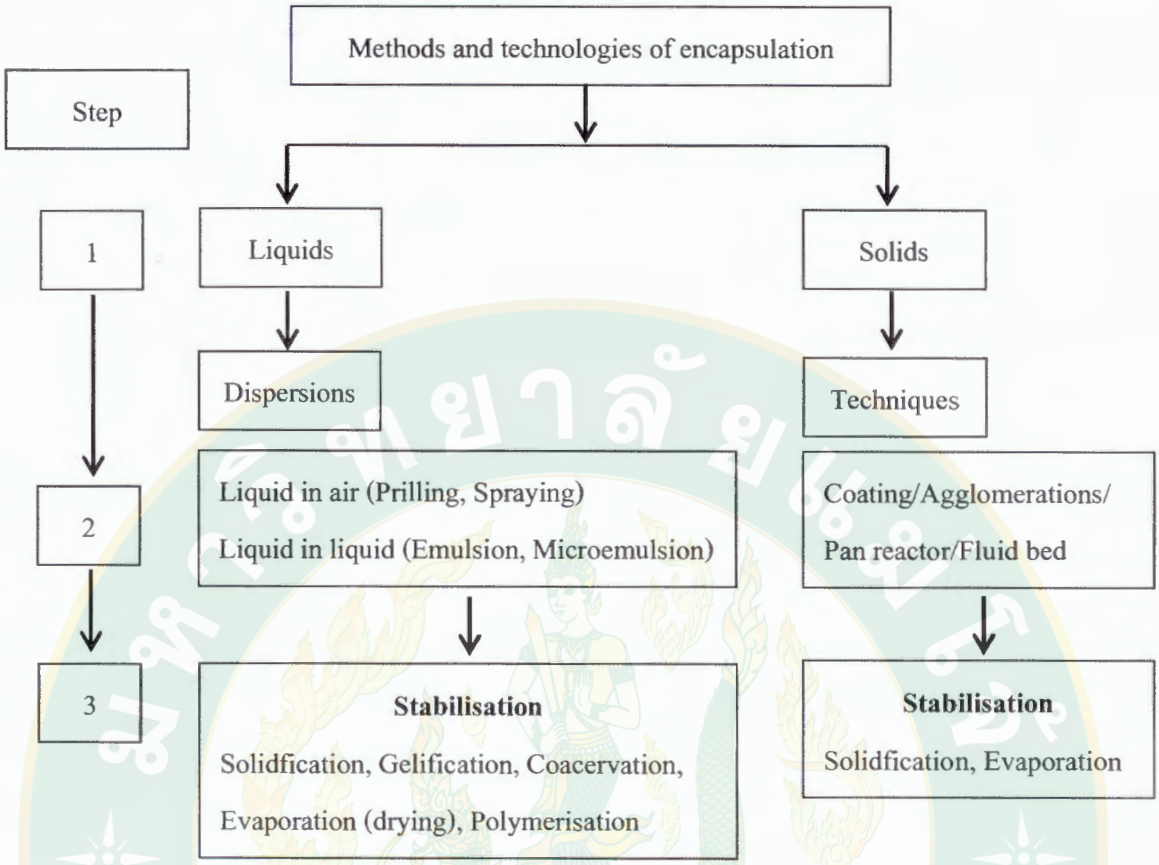


Figure 8 Encapsulation methods

2.1.6 Factors influencing encapsulation efficiency (Venkata et al., 2010)

The encapsulation efficiency of the microparticle or microcapsule or microsphere will be affected by different parameters such as solubility of polymer in the organic solvent, solubility of organic solvent in water, concentration of the polymer, ratio of dispersed to continuous phase, rate of solvent removal, interaction between core and polymer, solubility of core in continuous phase and molecular weight of the polymer.

2.2 *In situ* poly(urea-formaldehyde) encapsulation (Keller; Boh and Sumiga, 2013)

In situ polymerization is one of the chemical microencapsulation processes, taking place in oil-in-water emulsions. Encapsulation in a poly(urea-formaldehyde) shell has proven to be one of the most versatile and widely used encapsulation approaches within self-healing. This system will be referred to by the abbreviation UF. The UF encapsulation process is

emulsion-based and requires that the encapsulent be relatively immiscible in water. A detailed study of the encapsulation procedure and analysis of the microcapsule formation was given by Brown et al. The basic procedure for this encapsulation is shown in **Figure 9**.

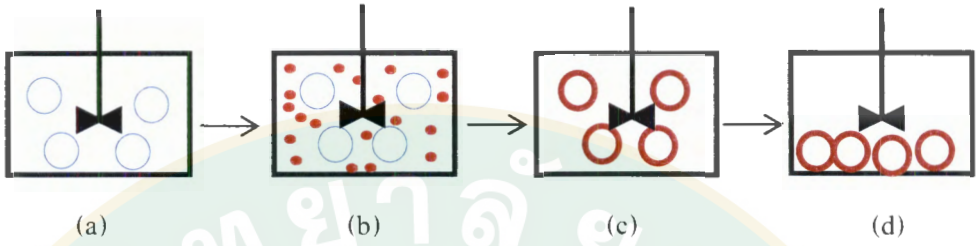


Figure 9 Schematic of the UF encapsulation procedure (a) Emulsification (b) Introduce shell material (c) Stirring and healing and (d) Filtration and drying

Microcapsules are produced by the polymerization and deposition of the UF polymer at the interface of the suspended emulsion droplet. Polymerization of the shell wall occurs in the aqueous phase until a critical molecular weight is achieved and the polymer phase-separates, depositing at the encapsulent-aqueous interface. The deposited polymer forms the shell wall of the microcapsule.

Two broad variations on this microencapsulation approach have been reported in the literature the *in situ* technique uses direct addition of urea and formaldehyde to the aqueous phase of the encapsulation bath. An emulsion of the encapsulent is established and urea and formaldehyde then added to the aqueous phase. Adjusting the pH to slightly acidic and heating the encapsulation bath initiates and completes the encapsulation. Capsules produced using this technique typically thick have thin shell walls, of the order of a few hundred nanometers, with a relatively thick porous layer of UF nanoparticles attached to the surface. However, if the encapsulent to wall material ratio is high enough, almost the entire mass of shell wall precursors can be consumed to produce shell wall, resulting in microcapsules with surfaces that are relatively smooth.

An indirect technique can also be used to produce UF microcapsules. In this approach, a prepolymerization of urea, melamine or phenol and formaldehyde is performed in a separate reaction vessel. The prepolymer is then added to the emulsified core material at the

point where the urea and formaldehyde would be in the *in situ* process. Capsules formed using the indirect procedure can have much thicker shell walls, up to several microns.

Emulsions for both encapsulation approaches are typically produced using physical stirring. The mixer blades induce a strong shear flow that breaks the encapsulent into small droplets. Capsule diameter is then directly relatable to stirring speed. While the mechanical agitation approach allows for the production of capsules as small as 20 to 30 microns in diameters, the ability to generate capsules of a single micron or less is generally beyond the scope of this technique. Several approaches to synthesizing sub-micron microcapsules utilized a combined ultrasonic dispersion and stirring technique to generate an emulsion with nanometer-sized droplets. Capsules were synthesized by the irradiating a low volume capsule batch with ultrasound and stirring to maintain the dispersion capsules of between one micron and several hundred nanometers in diameter.

Other researchers have used the Shirasu Porous Glass method to generate an emulsion with a narrow size distribution. Emulsions are produced by forcing an immiscible liquid through the membrane into a host liquid. These systems have demonstrated the capability to produce almost monodisperse liquid and gas emulsions. In an encapsulation setting, the membrane is used to form the droplets in the emulsion for an indirect UF encapsulation. One of the novel features of the reported procedure was the construction of a rotating membrane that served as both the emulsion source and the mechanical agitator that maintained the emulsion. While this approach yielded capsules that were within the capability of mechanical stirring, about 40 microns, the distribution of diameters was much narrow when to mechanically emulsified encapsulation procedures.

In addition to direct formation of microcapsules filled with a core of healing agent, the UF encapsulation procedure has also been successful in manufacturing capsules through a diffusion-based process. Encapsulation *via* this method is achieved by first producing either hollow or solvent-filled capsules. As mentioned previously, the indirect UF procedure produces capsules with relatively thick shell walls. These capsules are therefore far more stable to buckling collapse when compared to the capsules produced by the *in situ* technique and can be used to encapsulate air or gas bubbles entrained in the encapsulation bath. These bubbles can be produced by including a blowing agent in the encapsulation bath or by aggressively stirring an

encapsulation bath to entrain air. The hollow capsules can then be filled using a diffusion approach. Capsules are dispersed in the liquid of interest, which diffuses inward through the capsule shell wall, eventually fill the capsules as osmotic pressure equalize. This approach has proven useful for encapsulating highly reactive or water sensitive materials such as boron trifluoride diethyl etherate.

UF microcapsule has so far the largest commercial market in drug delivery, fragrance, carbonless copying paper and self-healing materials. Due to it have the properties of impermeable wall, stability, cheap and easy-making (Tian-Zhong, 2005).

2.3 Chemistry of urea-formaldehyde resin formation (Coner; Sánchez-Silva et al., 2012)

Urea-formaldehyde resins are formed by the reaction of urea and formaldehyde. The overall reaction of urea with formaldehyde is quite complex. The synthesis of a urea-formaldehyde resin takes place in two stages. In the first stage, urea is hydroxymethylolated by the addition of formaldehyde to the amino groups (**Figure 10**). This reaction is in reality a series of reactions that lead to the formation of mono-, di-, and trimethylolureas. Tetramethylolurea is apparently not produced, at least not in a detectable quantity.

The addition of formaldehyde to urea takes place over the entire pH range (**Figure 11**). The reaction rate is dependent on the pH. The rate for the addition of formaldehyde to successively form one, two, and three methylol groups has been estimated to be in the ratio of 9:3:1, respectively. The exact ratio, of course, is dependent on the reaction conditions employed in the addition reaction.

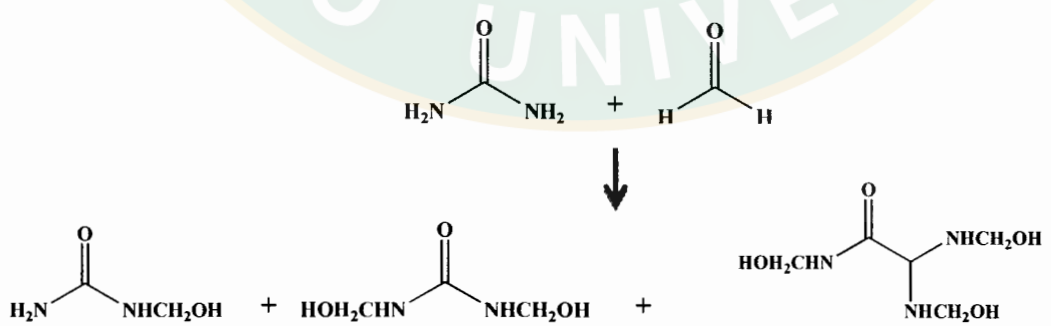


Figure 10 Formation of mono-, di-, and trimethylolurea by the addition of formaldehyde to urea

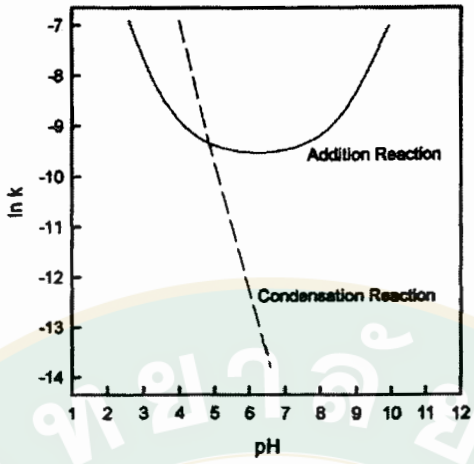


Figure 11 Influence of pH on the rate constant (k) for addition and condensation reactions of urea and formaldehyde (Corner, no date)

The second stage of urea-formaldehyde resin synthesis consists of the condensation of the methylolureas to low molecular weight polymers. The rate at which these condensation reactions occur is very dependent on the pH (**Figure 11**) and, for all practical purposes, occurs only at acidic pHs. The increase in the molecular weight of the urea-formaldehyde resin under acidic conditions is thought to be a combination of reactions leading to the formation of:

- methylene bridges between amido nitrogens by the reaction of methylol and amino groups on reacting molecules (**Figure 12a**);
- methylene ether linkages by the reaction of two methylol groups (**Figure 12b**);
- methylene linkages from methylene ether linkages by the splitting out of formaldehyde (**Figure 12c**); and
- methylene linkages by the reaction of methylol groups splitting out water and formaldehyde in the process (**Figure 12d**).

In general, the commercial production of urea-formaldehyde adhesive resins is carried out in two major steps. The difference between the pH profiles of the two stages of urea-formaldehyde resin synthesis is used in the production of urea-formaldehyde adhesive resins. The first step consists of the formation of methylolurea by the reaction of urea and formaldehyde under basic conditions with a pH of ~8-9. This step is carried out under basic conditions to allow the methylolation reactions to proceed in the condensation of the methylolurea.

In the second step, the reaction mixture is brought to the acid side, with a pH of about 5, and the condensation reactions are carried out until a desired viscosity is reached. Then, the reaction mixture is cooled and neutralized. Water is removed by vacuum distillation to give a resin with desired solids content (typically about 60-65%). Urea is often added in two, or sometimes more, steps. The initial addition of urea is made during the methylolation step, in which the formaldehyde-to-urea ratio is typically large (~ 1.6-2). Usually, the second addition of urea is made during the condensation step. The second and any subsequent additions of urea lower the final formaldehyde-to-urea ratio to the desired level.

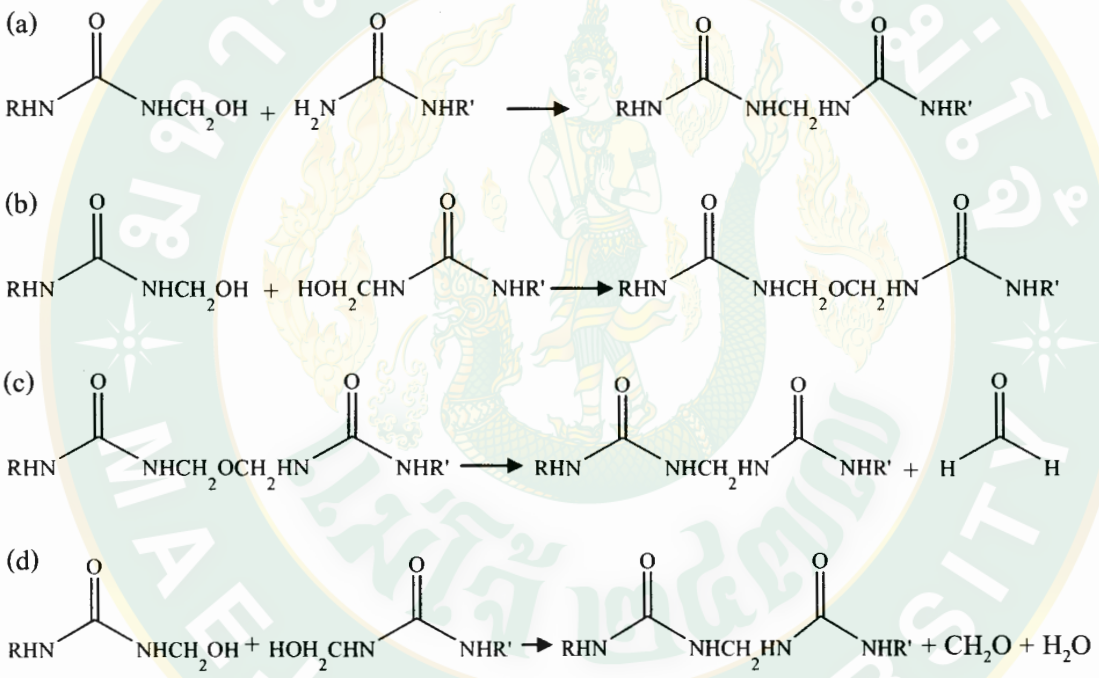


Figure 12 Condensation reactions of methylolurea to form (a) methylene bridges between amido nitrogens, (b) methylene ether linkages, and (c) and (d) methylene linkages. Reactions of these types produce higher molecular weight oligomers and polymers

Urea and formaldehyde are usually allowed to react in an acid medium to produce a water-insoluble crosslinked polymeric network with the aid of curing catalysts. Ammonium chloride and ammonium sulfate are the most widely used catalysts. A variety of other acids can be used as a catalyst, including formic acid, boric acid, phosphoric acid, oxalic acid, and acid salts of hexamethylenetetramine.

Resorcinol was used as a cross-linking agent under acid condition which reacted with formaldehyde to generate phenolic resin which was as a part of microcapsule wall.

2.4 Applications of microencapsulation

2.4.1 Agriculture (Dubey et al., 2009)

One of the most important applications of microencapsulated products is in the area of crop protection. Nowadays insect pheromones are becoming viable as a biorational alternative to conventional hard pesticides. Specifically, sex-attractant pheromones can reduce insect populations by disrupting their mating process. Hence small amounts of species- specific pheromone are dispersed during the mating season, raising the background level of pheromone to the point where it hides the pheromone plume released by its female mate. Polymer microcapsules, polyurea, gelatin and gum arabic serve as efficient delivery vehicles to deliver the pheromone by spraying the capsule dispersion. Further, encapsulation protects the pheromone from oxidation and light during storage and release.

2.4.2 Phamaceutics

One of the major applications of encapsulation technique is pharmaceutics, biomedical for controlled and sustained drug delivery. Potential applications of this drug delivery system are replacement of therapeutic agents (not taken orally today like insulin), gene therapy and in use of vaccines for treating AIDS, tumors, cancer and diabetes. Protein such as insulin, growth hormone, and erythropoietin (used to treat anemia) are example of drugs that would benefit from this new form of oral delivery. The delivery of corrective gene sequences in the form of plasmid DNA could provide convenient therapy for a number of genetic diseases such as cystic fibrosisand hemophilia. The spheres are engineered to stick tightly to and even penetrate linings in the gastrointestinal track before transferring their contents over time into circulatory system. Based on this novel drug delivery technique, Lupin has already launched in the market world's first Cephalexin (Ceff-ER) and Cefadroxil (Odoxil OD) antibiotic tablets for treatment of bacterial infections. Aspirin controlled release version ZORprin CR tablets are used for relieving arthritis symptoms. Quinidine gluconate CR tablets are used for treating and preventing abnormal heart rhythms. Niaspan CR tablet is used for improving cholesterol levels and thus reducing the

risk for a heart attack. Glucotrol (Glipizide SR) is an antidiabetic medicine used to control high blood pressure (Dubey et al., 2009).

2.4.3 Food industry

Variety of leavening system like baking soda, stable acids as well as vitamin C, acetic acid, lactic acid, potassium sorbate, sorbic acid, calcium propionate and sodium chloride can be encapsulate (Wilson and Shah, 2007). Commonly used encapsulated materials are citrus oils, mint oils, onion, garlic oils spice, and oleoresins. Citrus oils are very susceptible to oxidation due to sites of unsaturation in their mono and sesquiterpenoid structure. Encapsulated citrus oil, prepared by spray drying in malt dextrin matrix, has a greater stability than unprotected oils (Reineccius, 1988). Different wall components such as proteins (sodium caseinate and gelatin), hydrocolloids (Arabic gum) and hydrolyzed starches (starch, lactose, and maltodextrin) were utilized for encapsulation of extra-virgin olive oil by spray-drying (Patricia et al., 2010). Ginger oil powder prepared by using acacia gum as wall material by spray drying (Kadam et al., 2011). Curcumin microcapsules were prepared by spray-drying process using porous$_{\text{starch}}$ and gelatin as wall material (Wang et al., 2008).

2.4.4 Energy generation

Hollow plastic microspheres loaded with gaseous deuterium (a fusion fuel) are used to harness nuclear fusion for producing electrical energy. The capsules are multilayered. The inner layer, which compresses the fuel, is a polystyrene shell about 3 mm thick. Next is a layer of poly(vinyl alcohol) about 3 mm thick, that retards diffusion of deuterium out of the capsule. The outer layer (the ablator) is about 50 mm thick and consists of a highly crosslinked polymer made from 2-butene. During the fusion experiments, energy from high powered laser beams is absorbed by the surface of the microcapsule shell. As the outside of the shell (called ablator) burns off, the reaction force accelerates the rest of the shell inward, compressing and heating the deuterium inside. These results found in high densities and temperature in the center of the capsule leading to the fusion of deuterium nuclei to give tritium, helium and other particles releasing an enormous amount of energy. This process has been named as inertial confinement

fusion (ICF). Such ICF targets made of organic microcapsules have been in use since 1980s (Mishra et al., 2002).

2.4.5 Catalysis

Transition metal based catalytic processes are of vital importance to pharmaceutical, agrochemical and fine chemical industries. A vast proportion of such catalytic metal species are often expensive and toxic, thereby making operational handling potentially hazardous. Microencapsulation has recently been recognized as a useful alternative strategy to enable safe handling, easy recovery, reuse and disposal at an acceptable economic cost. Polyurea microcapsules due to their insolubility in aqueous and organic solvents, and resistance towards degradation have been used for encapsulation of different catalysts. Metal species such as palladium (II) acetate and osmium tetroxide have been encapsulated in polyurea microcapsules and used successfully as recoverable and reusable catalysts without significant leaching and loss of activity. It is thought that the urea functionality, which forms the backbone of the polymer, ligates and retains the metal species with in the polymeric matrix. Futuristic trend is towards incorporation of other chelating and ligating functional groups within the polyurea framework to study rate enhancement in such reactions, and trying other polymers for encapsulation (Ley et al., 2003; Ley et al., 2002).

2.4.6 Defense

One of the important defense applications of microencapsulation technology is in self-healing polymers and composites (Dubey et al., 2009). They possess microencapsulated healing agents embedded within the matrix and offer tremendous potential for providing long-lived structural materials. The microcapsules in self-healing polymers not only store the healing agent during quiescent states, but provide a mechanical trigger for the self-healing process when damage occurs in the host material and the capsules rupture. The microcapsules possess sufficient strength to remain intact during processing of the host polymer, yet rupture when the polymer is damaged. High bond strength to the host polymer combined with a moderate strength microcapsule shell is required. To provide long shelf life the capsules must be impervious to leakage and diffusion of the encapsulated healing agent for considerable time. These combined

characteristics are achieved with a system based on the *in situ* polymerization of urea-formaldehyde microcapsules encapsulating dicyclopentadiene healing agent. The addition of these microcapsules to an epoxy matrix also provides a unique toughening mechanism for the composite system. Such microcapsules have tremendous application in aerospace area for making self-repairable spacecrafts. Such self-healing spacecrafts open up the possibility of longer duration missions by increasing the lifetime of a spacecraft (Brown et al., 2003).

2.5 Microcapsulation in textiles

Microcapsules can contain perfumes, dyes, antimicrobials, phase change materials, vitamins and other substances. Different techniques can be used for applications of microcapsules to textiles such as padding, coating, spraying or immersion, impregnation with a transport of the textile through the basin, screen printing or inclusion of microcapsules into the textile fibres during the spinning process (Boh and Sumiga, 2013) or by incorporation in the fiber without modifying its sensation and color. For all these methods a binder is required. It may be acrylic, polyurethane, silicone, starch, etc. The role of binder is to fix the capsules onto the fabric and to hold them in place during washing and wear. A crosslinking agent is required for the impregnation of microcapsules into the fabrics. This is the component that forms the continuous film, adheres to the substrate, and holds the microcapsules in the fabric. Its function is to fix the microcapsules in the tissue and to keep them fixed during laundering. Several kinds of fabrics can be impregnated with microcapsules as silk, cotton or synthetic fibres (polyamide or polyester) (Slavica et al., 2006; Teixeira, 2010).

Commonly, the processes for applying microcapsules to textile materials can be described as follows:

1. Microcapsules are initially introduced in the textile material (without crosslinking agent) by means of using a dispersant to disperse the microcapsules around and through the textile material followed by the addition of a crosslinking agent to promote adhesion of the microcapsules to the textile material.

2. The microcapsules are applied during the finishing process of textiles fabrication using a foulard, in which the textile to be treated is impregnated using a finishing bath containing microcapsules, a softener and a self-cross-linking agent. Taking into account the type

of application the microcapsules have to fulfill specific requirements, such as resistance to abrasion and dry cleaning wash cycles. Microencapsulation has found several commercial applications as shown in **Figure 13**.

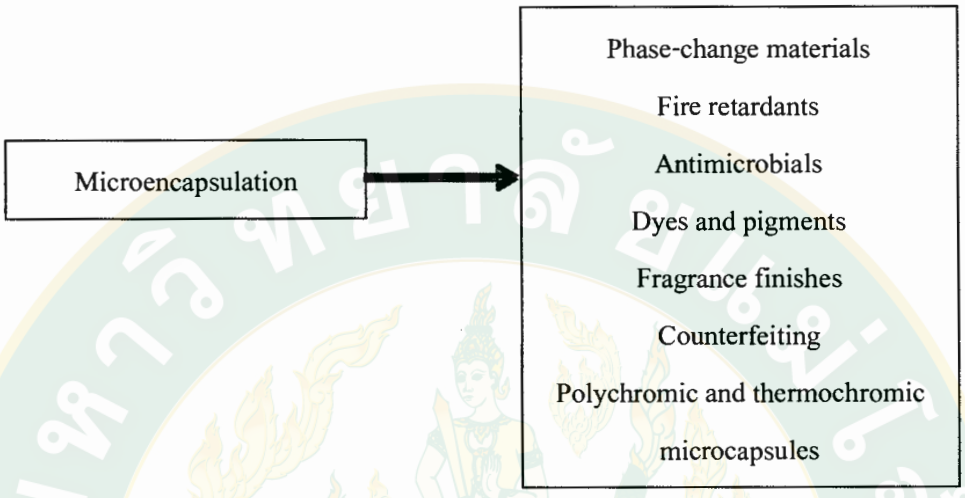


Figure 13 Microcapsulation in textiles

2.5.1 Cosmetic textiles

In cosmetic textiles, the major interest in microencapsulation is currently in the application of vitamins, essential oils, skin moisturizing agents, skin cooling agents, anti-aging agents etc. Focusing on the field of cosmetic textiles, the techniques of producing microcapsules containing essential oils and cosmetic substances have been studied extensively in the past (Cheng et al., 2008).

Yamato *et al.* prepared microcapsules comprising of active substances acting to improve the physiological conditions of human skin. The microcapsule would not break during production, but was gradually released when the textile structure was subjected to light pressure created by movement of the human body (US Patent, 1993).

Hak et al. investigated the flexibility of β-cyclodextrin as a protective wall. β-cyclodextrin was embedded onto cellulose fibres by using N-methylol-acrylamide. Benzoic acid and vanillin, which acted as an anti-bacterial agent and an aroma respectively, were encapsulated. It was claimed that the anti-bacterial activity was retained after 10 laundering cycles (Hak et al., 2000).

Wang and Chen developed aroma therapeutic textiles by using fragrance with β-cyclodextrin inclusion compounds and fixing them onto cotton fabrics with low temperature by using a conventional pad-thermofixed method. The fragrance release rates were greatly decreased and the results of sensorial evaluations showed that the performance of the fabric lasted for over 30 days (Wang et al., 2005).

2.5.2 Phase-change materials

The principle characteristic of these materials is the capacity to change their aggregation state within a limited temperature (from solid to liquid and vice versa), absorbing or expelling heat (Marinković et al., 2006). Microencapsulation technology was utilized in the early 1980s by the US National Aeronautics and Space Administration (NASA) with the aim of managing the thermal barrier properties of garments, in particular for use in space suits. They encapsulated phase-change materials (PCMs) (e.g. nonadecane) with the hope of reducing the impact of extreme variations in temperature encountered by astronauts during their missions in space. Ultimately the technology was not taken up within the space programme. However, the potential was recognised and after further development the work was licensed by the inventor, the Triangle Research and Development Co. to Outlast Technologies, based in Boulder, Colorado (Nelson, 2002). Outlast has exploited the technology in textile fibres and fabric coatings and PCM capsules are now applied to all manner of materials (Zubkova, 1995a,b; Colvin and Bryant, 1998), particularly outdoor wear (parkas, vests, thermals, snowsuits and trousers) and in the house in blankets, duvets, mattresses and pillowcases. As well as being designed to combat cold, textiles containing PCMs also helps to combat overheating, so overall the effect can be described as thermoregulation. The microcapsules have walls less than 1 μm thick and are typically 20–40 μm in diameter, with a PCM loading of 80–85%. The small capsule size provides a relatively large surface area for heat transfer. Thus the rate at which the PCM reacts to an external temperature changes is very rapid (Pause, 2000).

2.5.3 Fire retardants

Composite fire-resistant and lightweight building boards were patented (Adachi, 2005) containing a flammable substrate and a fireproofing Portland cement, which contained a foaming agent for generating an incombustible gas, a carbide layer precursor and microencapsulated carbide formation catalyst. Microencapsulated water was added to gypsum plaster boards, paints or thermal insulating materials Boh and Sumiga, 2008). Fire retardants have been applied to many textile products but in certain cases they can affect the overall handle, reducing softness and adversely affecting drape. Microencapsulation has been used to overcome these problems for example in fabrics used in military applications such as tentage (Kover et al., 1997). Others have incorporated the microencapsulated fire retardants during spinning of a polyester fiber for blending with cotton (Zubkova, 1997). Recently, di-ammonium hydrogen phosphate has been made in research focused on developing fabrics with microencapsulated fire retardants (Marinković et al., 2006).

2.5.4 Polychromic and thermochromic microcapsules

Color-changing technology has been around for a number of years, generally applied to novelty application such as stress testers, forehead thermometers and battery testers. New applications are now beginning to be seen in textiles, such as product labelling, and medical, security applications and in novelty textiles for purposes such as swimwear and T-shirts. There are two major types of color-changing systems: thermochromatic (which alter color in response to temperature) and photochromatic (which alter color in response to UV light). Microencapsulation of color-change material is produced to help protect these sensitive chemicals from the external environment. Now, the manufacturers are able to make dyes that change color at specific temperatures for a given application such as color changes can be initiated from the heat generated in response to human contact (Nelson, 2002).

Physiochemical and chemical processes such as coacervation and interfacial polymerisation have been used to microencapsulate photochromic and thermochromic systems. However, to obtain satisfactory shelf-life and durability on textiles, interfacial polymerization techniques are nearly always adopted. The most widely used system for microencapsulation of

thermochromic and photochromic inks involves urea or melamine formaldehyde systems (Aitken et al., 1996).

Several patents by Ma (2006a,b,c, 2007) described reversibly thermochromic cement- based materials, prepared by adding reversibly thermochromic microcapsules into a white Portland cement. Microencapsulated special thermochromic agents changed reversibly from blue, red or green colour at a lower temperature to white at a higher temperature. The system enabled a reversible change of colours: buildings were darker in winter, to absorb heat, and white in summer, to reflect the light energy.

2.5.5 Counterfeiting

In high added value textiles is great pressure to protect from illegal copying within the market. Microencapsulation can be used to help with this problem by offering a covert yet distinctive marking system. One example of this technology is that developed by Gundjian and Kuruvilla, 1999 of Nocopi Technologies. This system for combating textile counterfeiting utilizes microcapsules containing a color former or an activator applied to a thread or a label. The microcapsules adhere to the textile and depending on the type of chemical within the capsules can be detected at a later date to check authenticity. Detection may be achieved directly using UV light or more likely by using a solvent to break the capsules (Nelson, 2002).

2.5.6 Medical bandages

Microcapsulated essential oils of a white cedar were used in a mixture with a binder to protect building walls against generation of mildew (Arai, 2001). In a patent by Higashizaka (2002a,b, 2004), sustained release microcapsules containing hinokithiol were applied to protect leveling or base concrete in building construction against insects, bacteria, and corrosion, and to achieve a deodorising effect. A patent by Nishiguchi et al., (1998) described incorporation of microencapsulated fragrances, deodorants, antibacterial agents or insecticides into Calcium silicate shaped products for building interiors and exteriors.

2.6 Antimicrobial finishing textile (Cheng et al., 2008; Sathianarayanan et al., 2010; Umer et al., 2011; Keller)

Bacteria are often related to the notion of bad smell or disease and in textile industry the loss of useful properties of fabric often refers to microbiological decay of fibers. To prevent this problem the importance and demands of antimicrobial finishes continue to grow, especially for textiles for medical and technical use. Antimicrobial finishes can be applied to textiles also by microencapsulation. The release of active substances of microcapsules with antimicrobial agent is slow or sustained. Textiles with antimicrobial finishes are known in the market by different names, like Bacterbril, Biofresh, Terital, Trevia Bioactive, Amicor, etc.

Antimicrobial treatment for textile materials is necessary to fulfill the following objectives;

1. To avoid cross infection by pathogenic microorganisms.
2. To control the infestation by microbes.
3. To arrest metabolism in microbes in order to reduce the formation odor.
4. To safeguard the textile products from staining discoloration and quality deterioration.
5. Limit the growth of the bacterial colonies to their extinction.
6. To prevent the microbial attack on it and to prolong their useful life.
7. To protect the textile user against pathogenic or odor causing microorganisms.

The antimicrobial agents can be applied to the textile substrates by exhaust, pad-dry-cure, coating, spray and foaming techniques. The substances can also be applied by directly adding into the fiber spinning dope. Various methods for improving the durability of the finish include

- Insolubilisation of the active substances in/on the fiber.
- Microencapsulation of the antimicrobial agents with the fiber matrix.
- Treating the fiber with resin, condensates or cross-linking agents.
- Coating the fiber surface.
- Chemical modification of the fiber by covalent bond formation.

- Use of graft polymers, homo polymers and/or co-polymerization on to the fiber.

Mechanisms of antimicrobial finishes can be divided into two types based on the mode of attack on microbes. First, antimicrobial agent is slowly released from a reservoir either on the fabric surface or in the interior of the fiber. This leaching type of antimicrobial can be very effective against microbes on the fiber surface, or in the surrounding environment. Second, antimicrobial agent consists of molecules that are chemically bound to fiber surfaces. This agent can control only those microbes that are present on the fiber surface, not in the surrounding environment.

For testing antimicrobial activity, there are several methods have been developed to determine the efficiency of antimicrobial textiles. The tests to evaluate the antimicrobial activity are two categories, agar diffusion test (qualitative method) and dynamic shake test (quantitative method). Commonly, *Escherichia coli* (gram negative), *Staphylococcus aureus* (gram positive) and *Klebiella pneumonia* (gram negative) are used in testing methods.

The agar diffusion tests include AATCC 147-2004 (American Association of Textile Chemists and Colorists), JIS L 1902-2002 (Japanese Industrial Standards), SN 195920-1992 (Swiss Norm) and ISO20645:2004 (International Organization for Standardization). They are only qualitative, but are simple to perform and are most suitable when a large number of samples was to be screened for the presence of antimicrobial activity.

Bacteriostatic agar was dispensed in sterile petriplates.24 hour's broth cultures of the test organisms were used as inoculums. Using sterile cotton swab the test organisms were swabbed over the surface of the agar plates. The test fabrics and control was gently pressed in the center of the mat culture. The plates were incubated at 37°C for 18-24 hours.

The dynamic shake tests include ASTM E 2149-01 (American Society for Testing and Materials) and AATCC Test Method 100-1999 (American Association of Textile Chemists and Colourists). They provide quantitative values on the antimicrobial finishing, but are more time-consuming than agar diffusion tests. Specimens of the test material were shaken in a known concentration of bacteria suspension and the reduction in bacterial activity in standard time was measured. The efficiency of the antimicrobial treatment is determined by comparing the

reduction in bacterial concentration of the treated sample with that of control sample expressed as a percentage reduction in standard time.

2.7 Antimicrobial finishing agents

Many compounds are used to impact antimicrobial functionality to textiles, ranging from synthetic organic compounds such as triclosan, quaternary ammonium compounds, polybiguanides, N-halamines through to metals such as silver and naturally derived antimicrobials such as chitosan (Windler et al., 2013). The antimicrobial agents must kill or stop the growth of microbes and must maintain this property through multiple cleaning cycles or outdoor exposure. The antimicrobial agents must be safe for manufacturer to apply and consumer to wear. Moreover, they must be easily applied at the textile mill, should be compatible with other finishing agents and low cost. Here it is some of important antibacterial agents that are used in textile finishing.

2.7.1 Quaternary ammonium

Quaternary ammonium compounds are a chemical class of cationic surface active agents (Simoncic and Tomsic, 2010). Quaternary ammonium compounds seem attractive because their target is primarily the microbial membrane and they accumulate in the cell driven by the membrane potential (Shahidi and Wiener, 2012). To maximize efficiency, quaternary ammonium compound is used as monomeric link in the polymeric leash and poly(4-vinylpyridine) (PVP) is usually selected as the carrying polymer. Tiller et al. showed that the surfaces of commercial polymers treated with *N*-alkylated PVP groups were lethal on contact to both Gram-positive and Gram-negative bacteria, and it was also shown that *N*-alkyl chain of six carbon units in length was the most effective. In recent years, trialkyl ammonium chlorides have been reported to possess germicidal effect in dilute aqueous solutions. (Yao et al., 2008)

2.7.2 Triclosan

Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether), belonging to the chlorinated phenolic compounds which is applied in a various products as an antimicrobial and preservative (Dann and Hontela, 2011). It has a wide range of action against gram-negative and

gram positive bacteria. This compound also offers protection against mites and is used in acaricide (spray or powder) formulas, as well as in a solution (25% concentration) for the treatment of scabies. This compound is nontoxic. Due to its antibacterial properties, triclosan has found widespread use in a variety of consumer products including toothpastes, deodorants, soaps, polymers and fibers. (Allmyr et al, 2006)

2.7.3 Metallic salts

Numerous chemicals have been used to improve the antimicrobial activity of cotton textiles. Many heavy metals are toxic to microbes at very low concentrations either in the free state or in compounds. They kill microbes by binding to intracellular proteins and inactivating them (Shahidi et al, 2010). Although some other metals, such as copper, zinc and cobalt, have attracted attention as effective antimicrobial agents for textiles, silver is by far the most widely used in general textiles as well as in wound dressings. It has a MIC value of 0.05–0.1 mg/l against *E. coli*. Preparation of nano-sized metals and metal oxides, mainly silver (Ag), titanium dioxide (TiO_2), zinc oxide (ZnO) and cooper II oxide (CuO) has enabled the development of a new generation of biocides. Among these antimicrobial agents, silver has been widely used in many fields because it shows strong biocidal effects on many pathogenic bacteria.

Silver containing products are also interesting materials for wound repair applications. When metallic silver reacts with moisture on the skin surface or with wound fluids, silver ions are released, damaging bacterial RNA and DNA, thus inhibiting replication. Sustained silver release products have a bactericidal action and manage wound exudates and odour. In particular, Lansdown *et al.* have shown that silver aids healing in the sterile skin wound in rat models: silver treatment appeared to reduce the inflammatory and granulation tissue phases of healing and induce epidermal repair. (Blaker et al, 2004; Potiyaraj et al, 2007; Bingshe et al, 2007; Chen & Schluesener et al, 2008; Montazer et al, 2012; Ibrahim et al, 2012). The results of the counting test showed more reduction of survival of bacteria in the case of loading samples with metal salts.

Titanium dioxide (TiO_2) photocatalysts, as alternative materials to degrade organic substances for applications, have attracted much attention since the discovery of photo induced water cleavage on TiO_2 electrodes by Fujishima and Honda in the early 1970s. When

TiO_2 is exposed to ultraviolet light (λ<400 nm), holes (h_{vb}+) and excited electrons (e_{cb}-) are generated. The hole is capable of oxidizing water or hydroxide anions into hydroxyl radicals (UOH). UOH is known to be powerful, indiscriminate oxidizing agents to degrade a wide range of organic pollutants, including aromatics and aliphatics, dyes, pesticides and herbicides.

Zinc oxide nanoparticles have been shown to be useful antibacterial and antifungal agents (Ashjaran et al., 2014). Recently, the Raghupathi group also reported the properties of antibacterial activity against particles size. This report described the antibacterial activity of ZnO nanoparticles in the range from 212 nm to 12 nm particle size. The antibacterial activity of ZnO nanoparticles is inversely proportional to the size of the nanoparticles. (Raghupathi et al, 2011; Selvam and Sundrarajan et al, 2012)

2.7.4 Chitosan

Chitosan [poly-β-(1-4)-d-glucosamine], a cationic polysaccharide, is obtained by alkaline deacetylation of chitin, the principal exoskeletal component in crustaceans. As the combination of properties of chitosan such as water binding capacity, fat binding capacity, bioactivity, biodegradability, nontoxicity, biocompatibility, acceleration of wound healing and antifungal activity, chitosan and its modified analogs have shown many applications in medicine, cosmetics, agriculture, biochemical separation systems, biomaterials and drug controlled release systems.

Chitosan obtained from the shells of crabs, shrimps and other crustaceans, chitosan is a nontoxic, biodegradable and biocompatible natural polymer, and has long been used as a biopolymer and natural material in the pharmaceutical, medical, papermaking and food processing industries. Because of its polycationic nature, chitosan possesses a good antibacterial property against various bacteria and fungi through ionic interaction at a cell surface, which eventually kills the cell. Previous studies have shown that its antimicrobial activity is influenced by molecular weight, degree of deacetylation, temperature, pH and cations in solution. Because chitosan is one of the safest and most effective antibacterial agents, it has been widely applied for cotton and other textile antibacterial finishes. (Ye et al, 2005)

Several mechanisms were proposed for the antimicrobial activity by chitosan:

1. Polycationic structure of chitosan which can be expected to interact with the predominantly anionic components (lipopoly-saccharides and proteins of microorganism surface) resulting in changes in permeability which causes death of the cell by inducing leakage of intracellular components.

2. The chitosan on the surface of the cell can form a polymer membrane which prevents nutrients from entering the cell.

3. The chitosan of lower molecular weight enters the cell, binding to DNA and inhibits RNA and protein synthesis.

4. Since chitosan could adsorb the electronegative substance in the cell and flocculate them, it disturbs the physiological activities of the microorganism leading to death of the cells (El-tahlawy et al, 2005).

2.7.5 Cyclodextrin

Cyclodextrins are toroidal-shaped cyclic oligosaccharides with a hydrophilic outer surface and an internal hydrophobic hollow interior, which can entrap a vast number of lipophilic compounds into their hydrophobic cavity, depending on their size and molecular structure. The remarkable ability of cyclodextrins to include hydrophobic compounds has been exploited in several fields, spanning from pharmaceuticals to cosmetics, from food manufacturing to commodity.

2.7.6 Natural plants

The natural variants possess more potential for investigation because eco-friendly, least toxicity, suitability for next to skin innerwear and safe handling (Pannu, 2013). Plants produce a whole series of different compounds which are not particular significance for primary metabolism, but represent an adaptive ability of a plant to adverse abiotic and biotic environmental conditions. They can have a remarkable effect to other plants, microorganisms and animals from their immediate or wider environment. All these organic compounds are defined as biologically active substances and generally represent secondary metabolites. These secondary metabolites apart from determining unique plant traits such as color and scent of flower and fruit, characteristic flavor of species, vegetables, they also complete the functioning of plant

organism, showing both biological and pharmacological activity of a plant. Therefore, medicinal properties of plants can be attributed to secondary metabolites (Hartmann, 2008)

The phenolics and polyphenols are one of the largest groups of secondary metabolites that have exhibited antimicrobial activity. Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. Phenols are a class of chemical compounds consisting of a hydroxyl functional group (-OH) attached to an aromatic phenolic group. The site and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Geissman, 1963, as cited in Cowan, 1999).

Tanins, a group of polymeric phenolic substances, are found in almost every plant part such as bark, wood, leaves, fruits and roots. They are divided into two groups, hydrolysable and condensed tannins. In the plant tissue, tannins have been synthesized and accumulated after microbial attack. Their mode of antimicrobial action may be related to their ability to inactive microbial adhesins, enzymes, cell envelope transport proteins because of a property known as astringency.

The screening of plant extracts has been of great interest for discovery of new compounds effective in the treatment of bacterial infection. Plant extracts exhibit direct antibacterial activity showing effects on growth and metabolism of bacteria or/and indirect activity as antibiotic resistance modifying compounds which, combined with antibiotics, increase their effectiveness (Stefanović et al.).

Hein group studied the effect of antimicrobial agents from Aloe Vera gel on bleached cotton fabric. This study revealed that the antimicrobial activity of Aloe Vera gel treated fabric was excellent for inhibiting *Pseudo* and *E. coli* and good for inhibiting *B. aureus* and *B. pumilus* bacteria, but it could not inhibit the growth of *S. aureus* and *Candi* (Hein et al., 2013). Sharaf et al. applied propolis extract with glyoxal and $Al_2(SO_4)_3$ catalyst using the pad-dry method to produce a cotton textile with superior antibacterial activity, water repellant properties, and UV protection (Saraf et al., 2013). Plant extracts from the *Jatropha Curcas* leaf were used to treat cotton fabrics by the direct application method and antimicrobial activity was determined by an agar plate test. The results showed that the plant extract demonstrated a considerable zone of

inhibition to *S. aureus* and acted as a bactericide (Sudha et al., 2013). The extract of pomegranate (*Punica granatum*) was used for dyeing cotton fabric. The antimicrobial activity was assessed qualitatively by the disc diffusion method and AATCC 147, and was quantitatively tested by AATCC 100 against *E. coli* and *S. aureus*. Pomegranate extract displayed excellent antibacterial activity against both of the test organisms (Moussa et al., 2011). Moreover, acetic acid soluble material was isolated from the cell wall of *Mucor rouxii* DSM-1191 and applied on cotton fabrics. The results showed that *Mucor rouxii* DSM-1191 has excellent antibacterial activity against *E. coli* and *M. luteus* (Rajendran et al., 2011).

2.8 *Psidium guajava* Linn.

Psidium guajava Linn. (family Myrtaceae), is commonly called guava. *Psidium guajava* is a native plant throughout the South America, European, Africa and Asia (Gutiérrez et al., 2008). Many parts of this plant are used as a traditional medicine. The bark has been employed for treating diarrhea (Lee et al., 1012). The leaves are used for relief of cough, pulmonary disorders, wounds and ulcers. Moreover, guava has been demonstrated to have several biological activities such as antidiabetic, anticough, antioxidant, antibacterial and antispasmotic actions (Natitanon et al., 2010).

Figure 14 Guava leaves

Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids and the leaves of guava are rich in flavonoids and phenols (Venkatachalam et al., 2013). Leaves are the part of the plant that is most frequently used in the forms of decoction. Most of the pharmacological and chemical work has been carried out on the leaves (**Figure 14**).

Guava leaves contain tannins as well as β-sitosterol, flavonoids, triterpenoids, volatile oil. The main components of essential oil contain α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, cineol, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene. Flavonoids, and saponins combined with oleanolic acid include nerolidiol, β-sitosterol, ursolic, crategolic, and guayavolic acids. In addition, the leaves contain triterpenic acids as well as flavonoids; avicularin and its 3-L-4-pyranoside with strong antibacterial action, fixed oil 6%, 3.15% resin, and 8.5% tannin, and a number of other fixed substances, fat, cellulose, tannin, chlorophyll and mineral salts. In mature leaves, the greatest concentrations of flavonoids were found myricetin, quercetin, luteolin and kaempferol. Two triterpenoids, 20β-acetoxy-2α,3β-dihydroxyurs-12-en-28-oic acid (guavanoic acid), and 2α,3β-dihydroxy-24-*p*-*z*-coumaroyloxyurs-12-en-28-oic acid (guavacoumaric acid), along with six known compounds; 2α-hydroxyursolic acid, jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol D and β-sitosterol-3-*o*-β-D-glucopyranoside, have been isolated from the leaves of *Psidium guajava* (Gutiérrez et al., 2008).

Leaf extracts of *Psidium guajava* have been found to have antimicrobial activity against several bacteria, fungi, viruses and parasites, with proven ability to ameliorate diarrhoeal, gastroenteritis, dental plaque, acne, infantile rotaviral enteritis and even malaria suggesting wide antimicrobial activity (Gutiérrez et al., 2008). The aqueous and alcoholic extracts of guava (root as well as leaves) were found the inhibitory effects on the growth of *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Proteus* spp., *Shigella* spp. and *Escherichia coli*, causal agent of intestinal infections in humans were examined using the in vitro agar well diffusion method (Chah et al., 2006). In another study, aqueous and methanolic extracts of the leaves are effective inhibitors of growth spore formation, and enterotoxin production of *Clostridium perfringens* type A (Garcia et al., 2002). Moreover, *Psidium guajava* leaf and bark tinctures were subjected to *in vitro* sensitivity tests by serial dilution at concentrations ranging from 5% to 15% against six test dermatophytes, viz., *Trichophyton tonsurans*, *Trichophyton rubrum, Trichosporon beigelii*, *Microsporum fulvum*, *Microsporum gypseum* and *Candida albicans* (Dutta and Das, 2000).

Furthermore, guava leaves provide antioxidant and other affects providing beneficial protective properties to the heart and liver with an improvement in myocardial and

muscular function. In other animal studies guava leaf extracts showed anti-allergic, anti-inflammatory, analgesic, sedative, and depressant activity of central nervous system (CNS) (Gutiérrez et al., 2008).

For antioxidant activities, dried leaves of *Psidium guajava* were extracted with hot water. The total phenolic content in the extract was determined spectrophotometrically according to Folin–Ciocalteu's phenol method and calculated as gallic acid equivalent (GAE) (Ojan and Nihorimbere, 2004). Total phenolic content were found as 575.3±15.5 mg of GAE/g of dried weight material. The antioxidant activity of lyophilized leaf extracts was determined using free radical DPPH (2,2-diphenyl-1-picrylhydryzyl) scavenging. The results showed that ascorbic acid was a substantially more powerful antioxidant than the extracts from guava leaf. These antioxidant properties are associated with its phenolic compounds such as protocatechuic acid, ferulic acid, quercetin and guavin B, quercetin, ascorbic acid and gallic acid. Guava leaf extracts are a potential source of natural antioxidants (Thaipong et al., 2005).

2.9 *Sapindus rarak* DC.

Sapindus rarak DC. (*Sapindaceae*) or soap nut is a tall tree which originated in South East Asia and is now widely distributed in Asia (Wina et al., 2005). Its fruit pericarp is soft and brown color and it becomes dark brown when the pericarp is dried (**Figure 15**). Commonly, the pericarp contains foaming property in water thus it has been used as a natural soap or detergent for washing. *S. rarak* also has industrial application such as shampoos, soaps, tooth-paste and insecticides (Mahar et al., 2011). Moreover, *S. rarak* is used as herbal medicines, for example, removing lice from the scalp. The fruits are considerate for treatment a number of diseases like excessive salivation, pimples, epilepsy, chlorosis, migranes, eczema and psoriasis. Moreover, the powdered seeds are employed in the treatment of dental caries, arthritis, common colds, constipation and nausea (มะคำดีควาย, 2013).

In the pericarp mainly contains the saponins which are bioactive compounds. Saponins are secondary compounds found in several plants, a few marine animals, bacteria and insects. Saponins are consisted of two parts; glycone and aglycone. Glycone is a saccharide side group which is water-soluble. While, aglycone (known as sapogenin) is triterpenoid or steroidal

saponins which is liposoluble structure. From the composition of polar and non-polar structures of saponins, they exhibit soap-like in water (Vincken et al., 2007).

The sugars moieties of saponins can be D-glucose, D-galactose, D-fructose, 3-methyl-D-glucose, D-xylose, L-arabinose, L-rhamnose, L-fucose, D-apiose and D-chinovose, including D-glucuronic acid and D-galacturonic acid (Thakur et al., 2011).

Figure 15 *Sapindus rarak* DC. fruit pericarps

The chemical structure of saponins is high molecular weight glycosides which link to triterpene or steroidal glycone moiety. Generally, saponins are calssified into two groups based on the nature of their aglycone structure: steroidal saponins and triterpenoid saponins (Vincken et al., 2007).

Steroidal saponins consist of C_{27} spirotane skeleton, generally comprising of a six-ring structure (**Figure 16**). Some plant, the hydroxyl group in the 26-position is gaged in a glycosidic linkage and so the aglycone structure remains pentacyclic. This is referred to as a furostane skeleton (**Figure 16**). Some authors include glycoalcaloides with saponins having steroidal aglycone group. Triterenoid saponins consist of C_{30} skeleton which is comprised a pentacyclic structure (**Figure 16**). The majority of triterpenoid sapogenins belong to four basic skeleton: dammaranes, oleananes, ursanes and hopanes (Sparg et al., 2004; Chaieb, 2010).

In *Sapindus rarak* DC., arabinose is attached to hederagenin and rhamnose and xylose as sugar residues are attached to arabinose. The majority structure of *Sapindus rarak* DC. is hederagenin (aglycone) and mukurozi-saponin (**Figure 17**) (Wina et al., 2005).

The diversity and complexity of saponin structures arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone. It has been shown that the type of linkage and the sugar composition of

the saponin are directly related to their biological activity (Hart et al., 2008). The biological role of saponins is not completely understood. However, in plants, they was found that they acted as anti-microbial, fungicidal, allelopathic, insecticidal and moluscicidal, etc. activities in defense systems (Augustin et al., 2011).

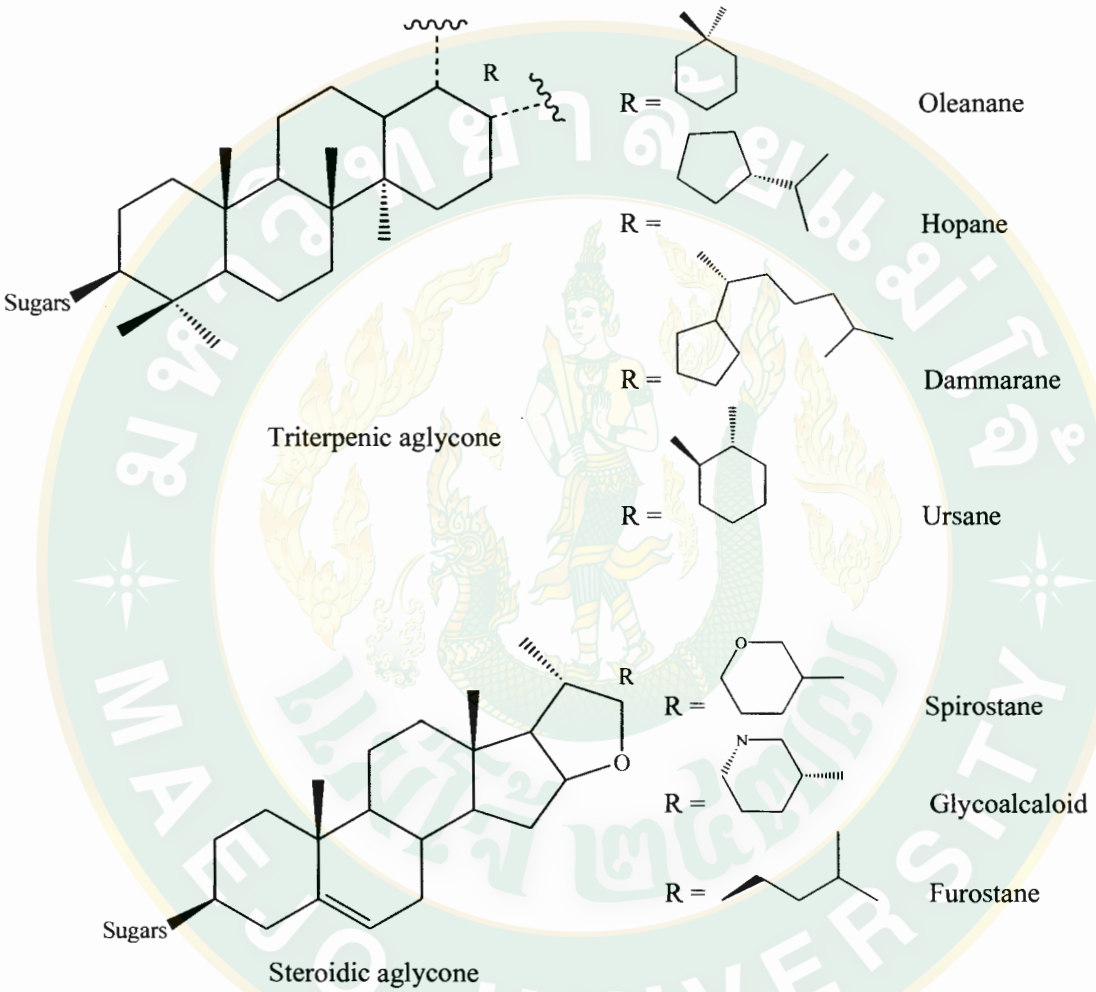


Figure 16 Different possible structures of saponin (Chaieb, 2010)

Chunet et al. extracted dried fruits of *Sapindus rarak* DC. with methanol and they were successful to isolate triterpene glycosides hederagenin 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopysanoside (sapindoside B) (**Figure 18**) and other three of its analogues by column chromatography. These compounds have been subjected to antimicrobial evaluations (Chunet).

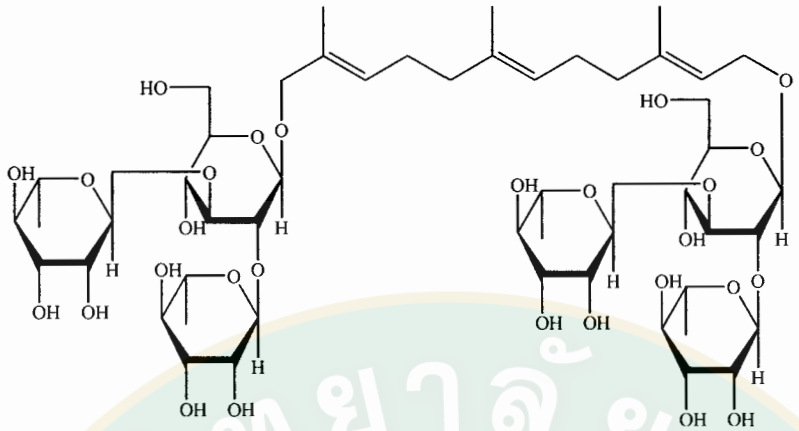


Figure 17 The structure of mukurozi-saponin (Asao et al., 2009)

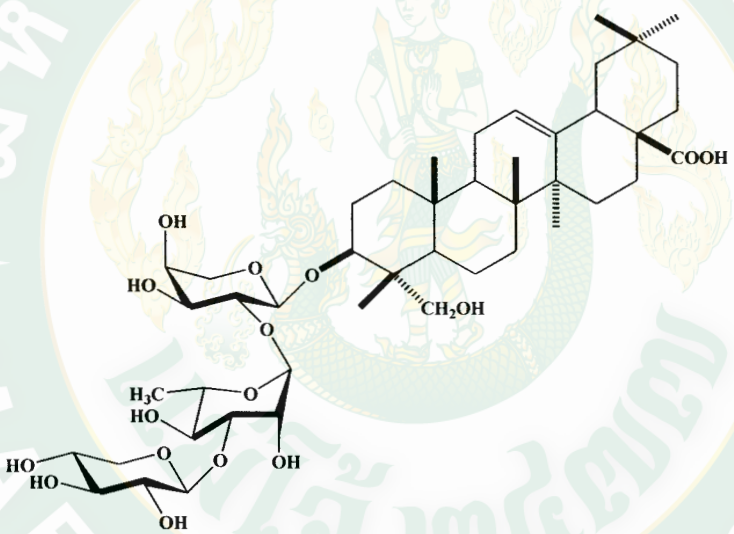


Figure 18 The structure of sapindoside B (Chunet, no date)

In additionally, Asao et al. reported a methanol extract from *Sapindus rarak* DC. pericarps. They were found three acylated oleanae-type triterpene saponins named rarasaponins IV, V and VI (**Figure 19**). The principle saponin constituents, hederagenin 3-*O*-α-D-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopysanoside and hederagenin 3-*O*-(3,4-di-*O*-acetyl-α-L-arabinopyranosyl)-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopysanoside showed inhibitory effects on plasma triglyceride elevation (Asao et al., 2009).

Furthermore, Kamra et al. reported the methanolic extract of *Sapindus rarak* DC. fruit resulted in 57% protozal numbers, 69% increased bacterial numbers, significantly reduced ammonia nitrogen, higher daily body weight gain and improved feed conversion efficiency (Kamra et al., 2006).

Addition of *Sapindus rarak* DC. to in vitro decreased significantly protozoal count, RNA concentration of Ruminococci and Chytridiomycetes (fungi) and xylanase activity in the rumen. Report of Thalib et al. showed *Sapindus rarak* DC. saponin extract kept the protozoal counts low when it was fed either daily or in interval of three days (Wina et al., 2006).

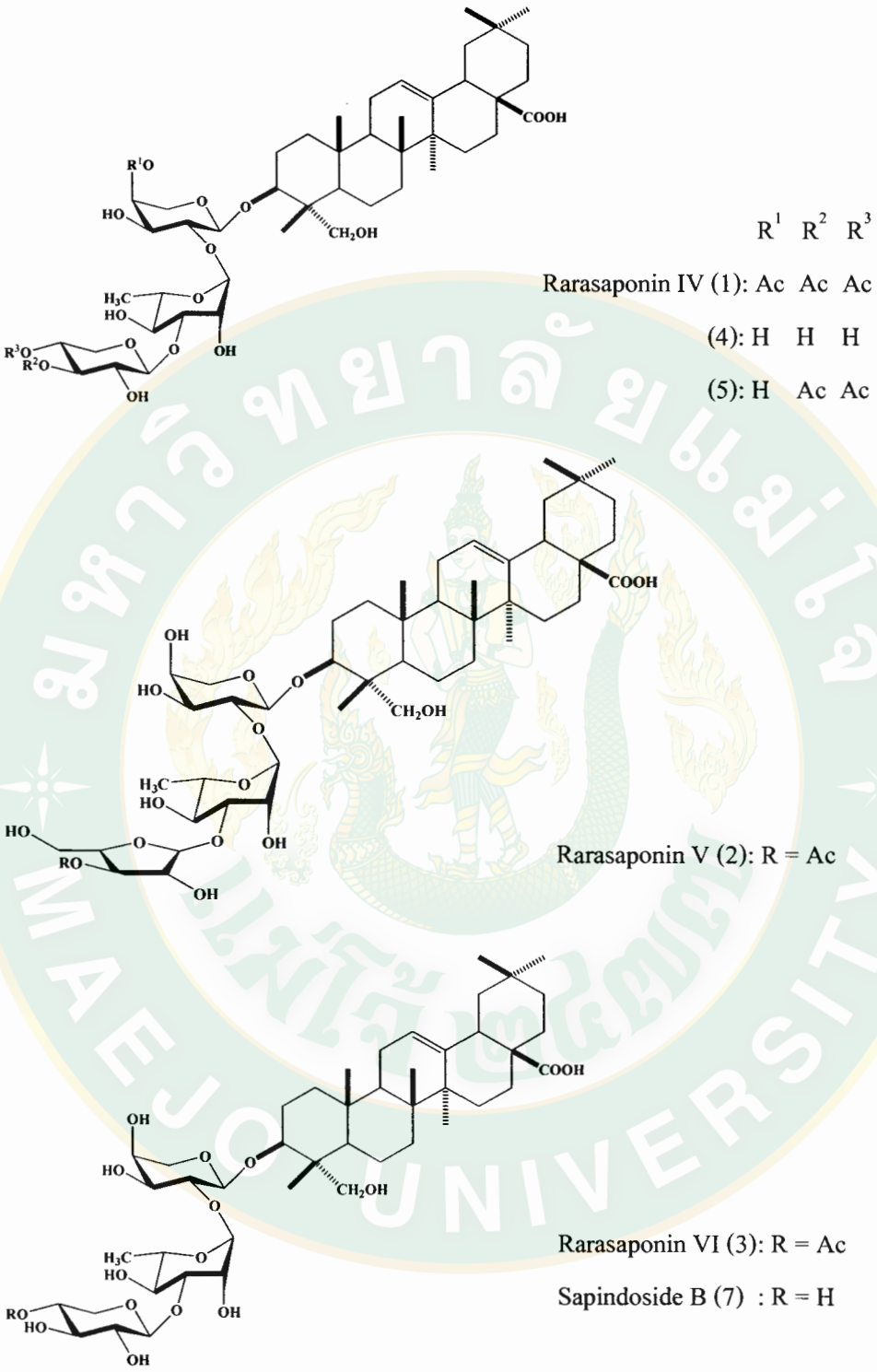


Figure 19 The structure of rarasaponins IV, V and VI (Asao et al., 2009)

CHARPTER 3

RESEARCH METHODOLOGY

3.1 Materials and instruments

3.1.1 Chemicals

Chemical	Assay	Company
Acetic acid	100.0%	Merck, Germany
Acetylacetone	99.0%	ACROS, Belgium
Ammonium acetate	98.0%	QRëC, Thailand
Ammonium chloride	99.0%	Rankem, India
Barium chloride dihydrated	99.0-100.5%	Loba chemie, India
Carboxymethyl cellulose (CMC)	-	Sigma Aldrich, Finland
Condensol	-	BASF, Germany
Ethanol	95.0%	Labscan, Thailand
	99.0%	Merck, Germany
Fixapret F-ECO	-	BASF, Germany
Folin-Ciocalteu reagent	-	CARLO ERBA, France
Formaldehyde	36.0%	Prolab, Belgium
Gallic acid	99.0%	Sigma Aldrich, Germany
Helizarin Binder CFF	-	BASF, Germany
Hydrochloric aicd	35.0%	Labscan, Thailand
Polyvinyl alcohol	-	Ajex Finchem, Australia
Resorcinal	99.0%	Hemidia, India
Sarsasapogenin	98.0%	Sigma, Mexico
Sodium carbonate	99.5%	Fisher, England
Sodium chloride	-	Ajax Finechem, Austraria
Sodium hydroxide	99.0%	Sigma, USA
Sulphuric acid	98.0%	Merck, Germany

Chemical	Assay	Company
Taninic acid	99.0%	Fluka, Germany
Triplicate soy agar	-	Himedia, India
Triplicate soy broth	-	Himedia, India
Urea	99.8%	Prolab, Belgium
Vanillin	99.0%	Sigma Aldrich, France
Zinc nitrate	-	Ajex Finchem, Australia

3.1.2 Instruments

Name	Model	Company
Adjustable micropipette	LAB-3 SERIES	Select Bioproduct, India
Autoclave	-	-, Thailand
Balance	AB 304-5	Mettler Toledo, Switzerland
	AND EK-300i	A&D Japan
Blender	-	Hanabishi, Japan
Digital camera	IXUS 105	Cannon, Japan
Freeze drier	-	Rabconco corporation, USA
FT-IR spectrometer	SpectrumRX I	PerkinElmer, England
Hot plate and stirrer	C-MAG H57	IKA, Germany
Mechanical stirrer	RW 20 digital	IKA, Germany
Optical microscope	BX41TF	Oympus, Japan
Oven	625G	Fisher scientific, England
Particle size analyzer	Mastersizer	MALVERN, England
pH meter	827 pH lab	Metrohm, Switzerland
Rotary evaporator	EYELA N-N series	Tokyo Rikakikai, Japan
Scanning electron microscope	LAB-3 SERIES	JEOL, Japan
Shaking bath	FTU-20D Tempunit	Techne, England
UV/Vis spectrophotometer	5410LV	Hitachi, Japan

Name	Model	Company
Thermometer	U-2001	-, China
Thermo gravimetric analyzer	-	Rigaku, Japan
Vacuum pump	Thermoplus TG 8120,	NDI, Germany
Ultrasonic bath	136H	NDI, Germany
Washing machine	AHIBA Easydye	Datacolor, USA
Water bath	SB-651	Tokyo Rikakikai, Japan

3.2 Test organisms

3.2.1 Bacterial strains

Eschericia coli (*E. coli*) and *Stepphylococcus aureus* (*S. aureus*) were obtained from Program of Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand. They were maintained on nutrient broth at 4°C in the refrigerator.

3.2.2 Culture medium

A. Broth/agar media are nutrient, trypticase soy and brian-heart infusion.

Nutrient broth:	Peptone	5 g
	Beef extract	3 g
	Distilled water	to 1000 ml

B. Dispensed in 10.0 ml amounts in conventional bacteriological culture tubes. Plugged and sterilized at 103 kPa (15 Psi) for 20 minutes.

C. For preparation of nutrient agar, 1.5% bacteriological agar was added to nutrient broth, the nutrient broth containing the culture was sterilized in 1000 ml borosilicate glass flasks and 15.0 ml of it was poured into petri dishes.

3.2.3 Maintenance of culture of test organisms

A. 200 µl Nutrient broth containing the culture was transferred into 10.0 ml nutrient broth by autopipette and the culture was incubated at 37±2 °C for 18-24 hr. The nutrient broth was prepared not more than two weeks.

B. Stock culture was maintained and stored at 4±1 °C and transferred once a month to fresh agar.

3.2.4 Preparation of 0.5 McFarland turbidity standard

A. McFarland 0.5 turbidity standard is available from various manufacturers. Alternately, the 0.5 McFarland was also prepared by adding 0.5 ml of a 1.175% (wt/v) barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) solution to 99.5 ml of 1% (v/v) sulfuric acid. The turbidity standard is then transferred into test tube, it was identical to those used to prepare the inoculum suspension. The McFarland standard tube was tightly closed to prevent evaporation and store in the dark at room temperature.

B. The accuracy of the density of a prepared McFarland standard was examined by using a spectrophotometer. For the 0.5 McFarland standard, the absorbance at a wavelength of 625 nm should be in the range of 0.08-0.13. Cell density of the adjusted suspension was approximately 10^8 colony forming units/ml (CFU/ml).

3.3 Preparation of natural extracts

Two plants including *Psidium guajava* L. leaf (guava leaf) and *Spindus rarak* DC. fruits (soap nut fruits) were studied in this work. The fresh leaf of guava was collected from Chiang Mai. Soap nut dried fruits were purchased from the local market in Chiang Mai. The step of plant extraction is shown in **Figure 20**

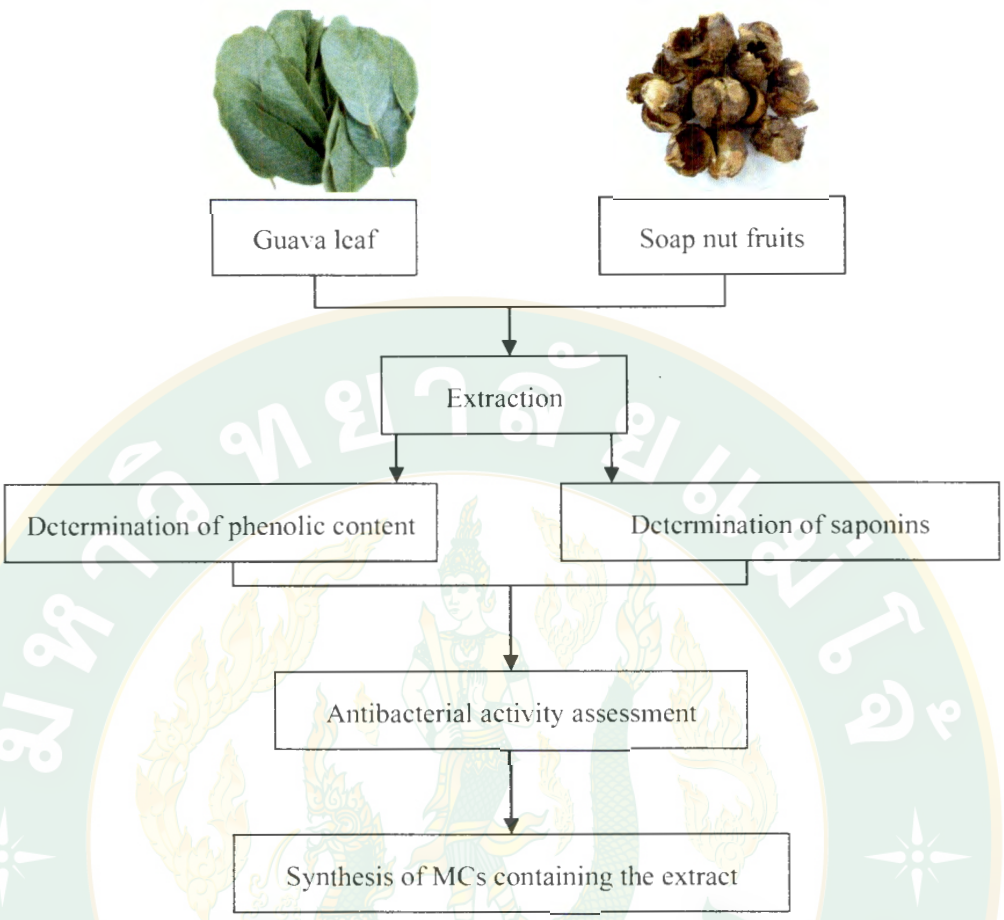


Figure 20 Scheme of preparation of plant extracts

3.3.1 Plants extraction

First, the fresh leaves of guava were washed to remove dirt and dried at 50 °C for 24 h in an oven. After they completely dried, the leaf was crushed into small pieces.

For the dried fruits of soap nut, they were peeled to remove the seed and then they were blended into small pieces. Next, the each plant sample was extracted with distilled water in a ratio of 1:15 at 60 °C for 1 h. The extracts were sieved through filtered cloth and followed with Whatman No.3 filter paper. The filtrates were concentrated by evaporating water and freeze-dried at -20 °C. Crude extracts were stored in 4°C for further application.

3.3.2 Determination of total phenolic content

Total phenolic compounds in the extracts from guava leaves were determined using Folin-Ciocalteu reagent assay which was modified from another investigation (Lee et al., 2012). Briefly, crude extracts were dissolved in distilled water at a final concentration of 0.1 mg/ml prior to test. An aliquot (0.5 ml) of samples was mixed with 0.5 ml of Folin-Ciocalteu reagent and 4.0 ml of 20%(wt/v) sodium bicarbonate solution, respectively. After 30 min maintenance at room temperature, the absorbance was measured spectrophotometrically at 760 nm. The experiment was run in triplicate. Tannic acid and gallic acid (0-10 μg/ml) were used as the standard for the calibration curve. The total phenolic content was expressed in terms of tannic acid/gallic acid equivalent (mg/g) of the extracts.

3.3.3 Determination of total saponins

The procedure was performed according to Shiau (Shiau et al., 2009). 0.5 ml of sample solution and 0.5 ml of 8 wt% vanillin solution in ethanol (fresh solution) were mixed together. After that, 5.0 ml of 72 wt% sulfuric acid was added and thoroughly mixed in an ice bath. The mixture was heated in water bath at 60°C for 10 min and then was cooled in ice bath. Finally, the solution was spectrometrically measured at 535 nm by UV-Visible spectrophotometer (U-2001, Hitachi, Japan). Sarsasapogenin (4.2-42.0 μg/ml) was used as standard for the calibration curve.

3.3.4 Determination of antibacterial activity of extracts

To assess the antibacterial activity of plant extracts, they were determined by agar well diffusion or plate hole diffusion method. First, preparation of nutrient agar according the step of **3.2.2** and the nutrient agar was poured into sterilized Petri dish for two-layered agar plate. After the first nutrient agar was left to set, the second layer was then prepared by nutrient agar plates were prepared by pouring of media into Petri dishes. The lower agar consisted of approximately 10.0 ml and the upper layer consisted of approximately 5.0 ml agar inoculated with bacteria of $1x10^5$ – $3.0x10^5$ CFU/ml. Whereby, the grown cultures which were prepared according to the step of **3.2.3.A** were transferred to 100.0 ml borosilicate glass flask containing the nutrient agar with until the turbidity of solution was equivalent to 0.5 McFarland standard.

Then, 1.0 ml of the cultures were then serially diluted 3 fold to obtain an approximately final concentration of $1x10^5$ – $3.0x10^5$ CFU/ml. The agar plates were allowed to solidify for 20 min and holes/wells of 10 mm diameter were punched into the agar with the help of flamed cork borer. 0.2 ml of the plant extract (500 mg/ml) was filled in each hole. After incubation at 37°C for 24 hr, the zone of inhibition was observed and recorded. The experiments were done in duplicate.

3.4 Urea-formaldehyde microcapsules containing extracts

The steps of urea-formaldehyde microcapsule preparation are shown in **Figure 21**.

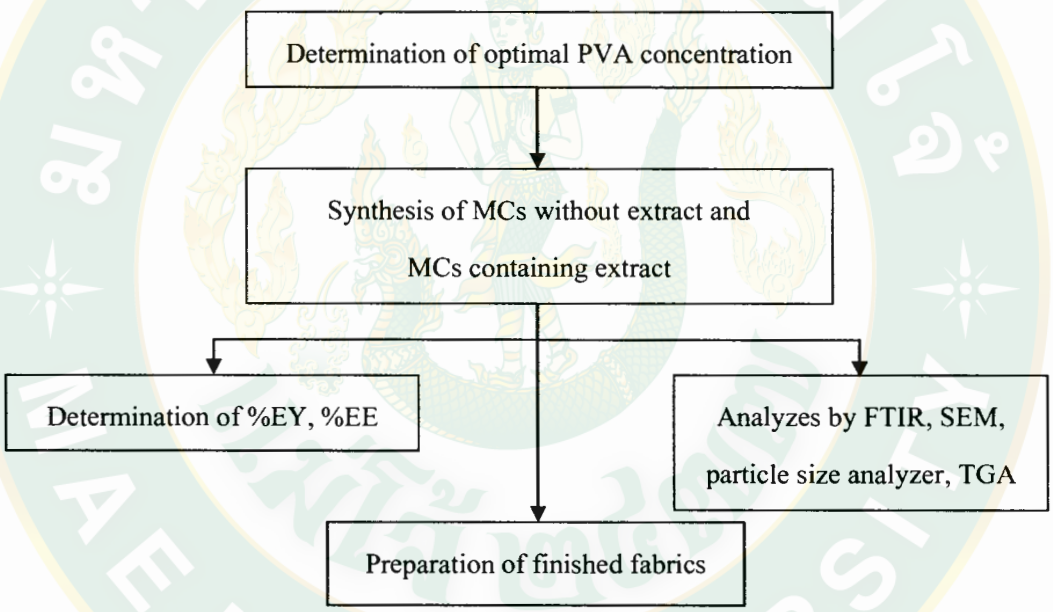


Figure 21 Scheme of studying MCs containing extract

3.4.1 Determination of optimal PVA concentration

PUF MCs were prepared by in situ polymerization according to the procedure of Suryanarayana (Surynarayana et al., 2008) with modifications. Firstly, 5 g of urea, 0.5 g ammonium chloride and 0.5 g resorcinol were dissolved in 260 ml distilled water. Secondly, 10 ml of aqueous solution of PVA was added into the solution and the pH of the solution was adjusted to 3.5 with 5% hydrochloric acid. PVA solution was varied to be 1%, 3% and 5%wt/v. After 12 ml of 36% wt formaldehyde solution was added, the reaction was constantly stirred at

800 rpm and heated at 55°C for 4 hr. The obtained MCs were subsequently filtered with Whatman No.3 paper under a vacuum and washed with distilled water. Finally, the prepared MCs were dried at room temperature and stored under a vacuum condition.

3.4.2 Preparation of microcapsules containing extracts

PUF MCs containing extract were prepared followed with the step of **3.3.1**. Firstly, 5 g of urea, 0.5 g of ammonium chloride, 0.5 g of resorcinol and 260 ml of distilled water were mixed in 500 ml beaker. Then, the mixture was added with 10 ml of 5%wt aqueous solution of polyvinyl alcohol (PVA). After the solution pH was adjusted to 3.5 by 5 wt% hydrochloric acid solution, the extract (guava leaf extract/soap nut extract; weight ratio of extract to urea: 0/5, 1/5, 2/5, 3/5, 4/5 and 5/5) was loaded in the mixture and left for 10 min for stabilization. Then, 12 ml of 36 wt% formaldehyde solution was added. The reaction was constantly heated at 55°C and carried out under stirring at 800 rpm for 4 hr (**Figure 22**). The prepared microcapsules were filtered and the supernatant was collected to determine %EE (**3.3.3**). Last, microcapsules were thoroughly washed with distilled water and dried at room temperature and stored under vacuum.

For the preparation of MCs without the extract, the process was done according to the above methodology, but the extract did not load into the mixture of urea, ammonium chloride and resorcinol. The procedure was completed by the mixture reacted with formaldehyde without adding the extract.

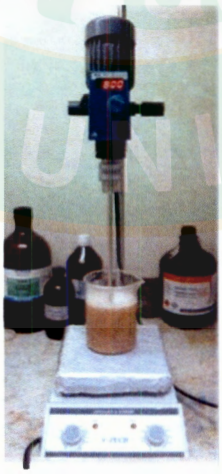


Figure 22 The synthesis of MCs

3.4.3 Determination of encapsulation yield and efficiency encapsulation

Encapsulation yield (EY) (Banerjee et al., 2013)

Encapsulation yield was calculated as the percentage of weight of microcapsules obtained at the end of the process and the weight of initial materials added including guava leaf extract, urea, resorcinol, PVA and formaldehyde as shown in equation (**3.1**).

$$\%EY = \frac{\text{wt of obtained microcapsules}}{\text{wt of total materials}} \times 100 \qquad (3.1)$$

Encapsulation efficiency (EE)

Encapsulation efficiency was calculated as the percentage of weight of the extract was entrapped in microcapsules and weight of the extract was used.

$$\%EE = \frac{w_1 - w_2}{w_1} \times 100 \qquad (3.2)$$

when w_1 = wt of extract used for preparation microcapsules

w_2 = wt of extract left in supernatant after preparation of microcapsules

3.4.4 Analyses of microcapsules

First, the composition of microcapsules and extract were obtained to identify the chemical structure using a FTIR spectrometer (Perkin Elmer), was prepared by grinding the sample with a potassium bromide (KBr) and analyzed in KBr pellet form. The test samples were guava leaf extract, soap nut extract, MCs without extract, MCs containing guava leaf extract and MCs containing soap nut extract whereby MCs containing extract at 5/5 weight ratio of extract to urea.

Second, the morphology and surface of microcapsules was observed by scanning electron microscope (SEM, 5410LV JEOL). MC samples were dispersed in distilled water and sonicated for 30 min. After that, one drop of MCs dispersion was placed on the surface of a double-faced black adhesive tape that attached to a stainless steel stub and dried at 50 °C for 1 hr. The samples were sputtered with a thin layer of gold. The sample was sputtered with a thin layer of gold.

Third, the MC particle size distribution was carried out using particle size analyzer (Mastersizer, MALVERN) based on light scattering apparatus (**Figure 23**). This equipment has a sample dispersion accessory which allows the system to be used for particle-in-liquid particle sizing. The dispersion unit consists of an electronic motor that drives a stirrer and an impeller in the tank to provide a simultaneous stirring and pumping action. Distilled water was used as dispersant since it does not have interaction with the particles. MCs without extract and MCs containing extract at 1/5, 3/5 and 5/5 weight ratio of extract to urea were analyzed to compare the distribution.

Last, the extracts, MCs without extract and MCs containing extract at 1/5, 3/5 and 5/5 weight ratio of extract to urea were analyzed using thermogravimetric analyzer (TGA, Thermoplus TG 8120, Rigaku, Japan). The samples were analyzed at a heating rate of 10 °C/min from 27-500 °C in nitrogen environment.

Figure 23 The image of particle size analyzer

3.5 Cotton fabric finished with microcapsules

3.5.1 Preparation of cotton fabric finished with microcapsules

Both the extracts and the MCs containing extract were applied to cotton fabric using the printing method with cross-linking agent. 50 g/l of guava leaf extract/MCs was mixed with 2% wt of CMC, 50 g/l of Helizarin, 50 g/l of Fixapret F-Eco, 20 g/l of Condensol and 10 g/l of zinc nitrate. The cotton fabric was coated with the mixture using a printing machine (Kidd + Zigrino) (**Figure 24**). The fabrics were dried at 105 °C for 90 seconds and cured at 175 °C for 45

seconds by heating oven (**Figure 24**). The fabrics were rinsed with water before further testing. The modified surface of the finished fabric was observed using optical microscope (BX41TF, Olympus) and SEM (5410LV, JEOL). Moreover, they were tested of wash durability, antibacterial activity and determined for free formaldehyde amount.

Figure 24 The images of printing and heating oven for fabric drying and curing

3.5.2 Analyses of finished cotton fabrics

Optical microscope (**Figure 25**) and SEM techniques were used to investigate the surface morphology of different cotton fabrics before and after finished with the extracts of guava leaf and soap nut and MCs containing guava leaf extract/soap nut extracts. For the observation by optical microscope, a small piece of fabric sample was placed on glass slide and the objective was focused with the 40x objective for 400x magnification. Finally, the detail of fabric was captured and recorded.

Figure 25 The image of optical microscope

Furthermore, the fabric samples were glued to stainless steel stub with double-faced black adhesive tape and sputter coated with gold. Sample was investigated and digitally imaged at 500 kV accelerating voltage by scanning electron microscope (SEM).

3.5.3 Testing of wash durability

Washing was carried out according ISO 105-C01:1989 as standard method by using a standard soap (5 g/l) and kept the materialistic liquor ratio at 1:50 (weight of g fabric: volume of soap solution). The specimens were carried out in washing chamber which containing soap solution at 40±2 °C for 30 minutes and speed at 40±2 rpm as one washing cycle (**Figure 26**). The process was done following by rinsing, washing and drying. After drying, the specimens were assessed for antimicrobial activity using AATCC 147 procedure after 1st and 5th washing cycles.

Figure 26 The images of washing shamble, standard soap and washing machine

3.5.4 Assessment of antibacterial activity

All finished fabrics were qualitatively determined the antibacterial activity using agar diffusion method against *E.coli* and *S.aureus*. After that the testing were also tested using AATCC 147-2004 method to confirm the efficient of finished fabrics.

Disc diffusion method (SN 195920-1992)

Cotton fabric was used as control in this work. Unfinished cotton fabric and cotton fabrics finished with guava leaf extract, MCs containing guava leaf extract, soap nut extract and MCs containing soap nut extract were cut into a square with a diameter of 20 mm. After the agar nutrient medium was poured into sterilized Petri dishes, the agar was allowed to solidify. The test organisms with a concentration of 10^8, 10^7 and 10^6 CFU/ml were swabbed over the surface of agar plates and were left to dry for 5 min. The cotton fabric and finished fabrics were placed on the surface of medium and the plates were incubated at 37 °C for 24 hr. Last, the antibacterial activity of fabrics was demonstrated by the observation of the inhibition zone. The clear zone appeared around the disc indicated the presence of antibacterial activity. The experiment was performed in a duplicate.

AATCC Test Method 147-2004

Finished fabric was assessed the antibacterial activity according AATCC147-2004 standard (Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method, American Association of Textile Chemists and Colorists, RTP, NC.) with modification. The fabrics were cut in rectangular shape with 10x50 mm. Briefly, one loop of bacteria without refilling was streaked 5 lines on the surface of the nutrient agar plates. Cell concentration of bacteria in this experiment was 10^8 CFU/ml. Then, the fabric samples were placed over the culture incubated agar. After incubation at 37 °C for 18-24 hr, a streak of interrupted growth underneath and along the side of the test fabric used to evaluate the antibacterial effectiveness of the fabric.

3.5.4 Determination of free formaldehyde

Method for the determination of free formaldehyde is 2,4-pentanedione method according to BS EN ISO 14184-1:1999 part I Annex A (Textiles-Determination of Formaldehyde-Part 1, 1998). Briefly, 0.5 g of microcapsule samples was firstly put into 250 ml flask and followed by 20 ml of deionised water. The flask was covered with stopper and heated in a water bath at 40 °C for 1 h with shaker. Then, the warm solution was filtered into another flask through a Whatman filter. After 5 ml of solution sample was transferred into a tube, 5 ml of acetylacetone reagent solution was added into the tube and covered with stopper and shake it. The mixture was kept first in a water bath at 40 °C for 30 min and then was left at room temperature for 30 min. Blank reagent was prepared by adding 5 ml of acetyl acetone reagent solution to 5 ml of deionised water and treated it the same way. The absorbance of solution was measured at 415 nm using a spectrophotometer (U2001 Hitashi, Japan). Formaldehyde solutions (0.15-3.00 μg/ml) were prepared as the calibration solutions. Each test was performed in triplicates.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Extract preparation

Guava leaf and soap nut pericarps were extracted with hot water (1:15 ratio (wt/v)). Crude extracts were concentrated, freeze dried at -20 °C and stored at 4 °C for further applications. The extracts from two plants are shown in **Figure 27**. Guava leaf extract was dark brown and soap nut extract was light brown.

(a) (b)

Figure 27 The extract of (a) guava leaf and (b) soap nut

Next, the extract of guava leaf was determined for total phenolic content by Folin-Ciocalteu assay using gallic acid and tannic acid as the standard. A calibration curve was prepared from a plot of absorbance of solution versus concentration of gallic acid or tannic acid and the total phenolic amount were determined from the curve as shown in **Appendix A**. The result of the oxidation of phenols in alkaline solution by the yellow molybdotungtophosphoric heteropolyanion reagent, Folin-Ciocalteu reagent was blue pigments, molybdotungstophosphate blue (Lee et al, 2012). The depth of blue color depended on the qualitative composition of phenolic content. Total phenolic content in guava leaf extracts were 28.1 mg GAE/g and 38.8 mg TAE/g of guava leaf extract. The hot water extraction of phenolic compounds in guava leaf in this study was the effective method when compared to other research groups. In the research of Venkatachalam et al. (Venkatachalam et al., 2012), it was reported that the aqueous extract of guava leaf exhibited the total phenolic content as 7.38±0.09 mg GAE/g of extract. The tannin content in guava leaf was estimated to be 104±1.73 mg TAE/g of extract. Additionally, the previous study of Natitanon et al. (Natitanon et al., 2010) was revealed that hot water was the

better solvent than ethanol and ethyl acetate for extraction of phenolic compounds in guava leaf. The extracts of three different leaf maturities: young, middle and old age leaf contained the total phenolic content as 6.27±0.23, 2.43±0.11 and 3.72±0.22 mg GAE/g of extract, respectively.

The extract of soap nut was quantified for saponin content according to vanillin-sulfuric acid assay as 246.0 mg/g extract and sarsasapogenin solution was used as calibration solution. Calibration curve of sarsasapogenin was shown in **Appendix B**. The reaction of aromatic aldehydes and vanillin in strong mineral acids, sulfuric acid, gave a red, blue or green colored product with aglycones. Dehydration was occurred forming unsaturated methylene groups with aldehydes. Terpenoid saponins tended to produce a pink or purple shade and steroid saponins gave a blue-green coloration (US patent, 2006).

Subsequently, the extracts from guava leaf and soap nut were screened for antibacterial activity by agar diffusion test. Gentamycin was positive control in this study. The inhibition zone was observed (as seen in **Figure 28**). The inhibition was observed against *S.aureus* with a zone of inhibition around guava leaf extract and soap nut extract of 18.0 mm diameter. Whereas the guava leaf extract and soap nut extract exhibited a zone of 15.0 mm and 14.0 mm inhibition for *E.coli*.

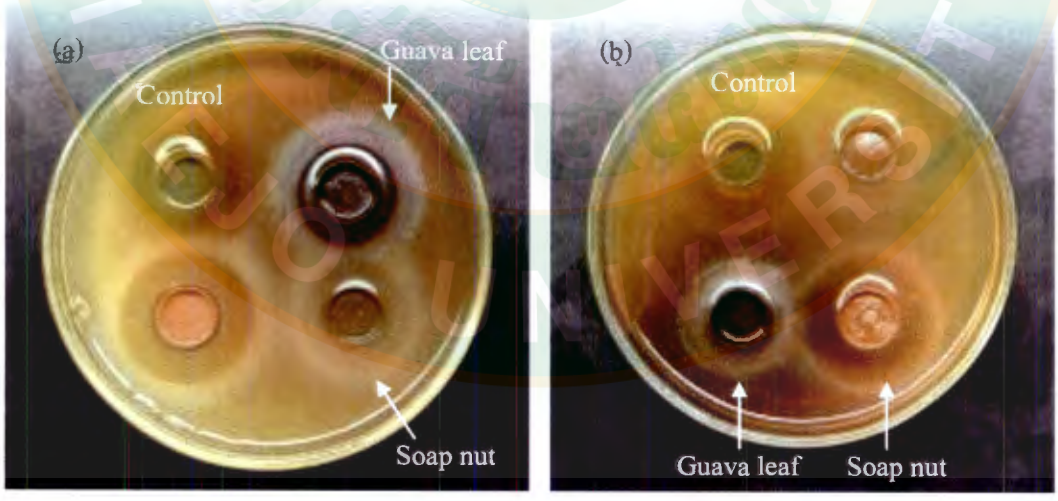


Figure 28 Antibacterial activities of guava leaf extract and soap nut extract by agar diffusion method (a) *S.aureus* and (b) *E.coli*

4.2 Preparation of MCs

MCs were prepared by in situ polymerization using urea and formaldehyde as a wall materials. First of all, encapsulation of guava leaf extract in a PUF shell was carried out when the pH becomes acidic, was heated to 55 °C, and reacted with urea and formaldehyde resulting in a PUF. In the initial step of polymerization, the urea-formaldehyde molecule was rich in polar groups and was water compatible. The product of this step was called methylol urea. Next, the number of polar groups was gradually reduced as the molecular weight of the polymer increases. Finally, the hydrophilicity of the PUF molecule was reduced leading to separation from the aqueous phase, and droplets of MC powder were received. MCs without extract showed white color (as in **Figure 29**).

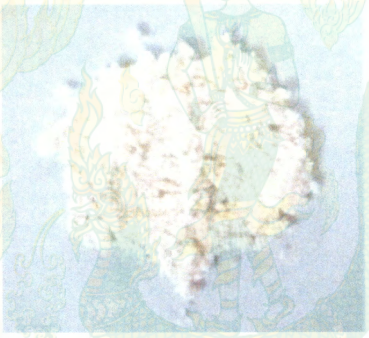


Figure 29 MCs without extract

To determine the optimal concentration of PVA solution for preparation of PUF MCs, PVA solution was varied in three different concentrations, 0, 1, 3 and 5 %wt/v. PVA is responsible as emulsifier to form with urea and formaldehyde resulting in micelle formation in water. Hence, the influence of the concentration of PVA on the synthesis of MCs was examined. Different concentrations of PVA solution (1, 3 and 5 %wt/v) were used with urea and formaldehyde and in this series experiments the extract was not yet loaded in the reaction. Comparisons of the shape and the size of the obtained MCs are shown in **Figure 30**. MCs without adding PVA had the largest particles, but also small particles were found. However, MCs were prepared by adding 1% PVA solution in the reaction had an irregular shape. Most MCs were found to have small particle sizes attached to form an irregular shape. This may have been

caused by the concentration of PVA in the reaction but did not fully generate an emulsion during the reaction. After that, the concentration of PVA solution was increased to 3% and then to 5% and resulting the size of MCs was found to be smaller and a spherical shape. However, it was found that if a 5% PVA solution was used in the reaction, it gave the smallest MC particle size and also had the optimal concentration for PUF MCs synthesis. This experiment was in agreement with the report of Park et al. (Park et al., 2001). They prepared UF MCs containing lemon oil using different emulsifiers, gelatin, Span 80, PVA and sodium dodecyl sulfate. They revealed that the MCs prepared using PVA, the particle size was small. Moreover, they found that small particle size of MCs with increasing emulsifier content up to 5% was due to hydrolysis of the emulsifier and water and interaction of micelles with increasing emulsifier content.

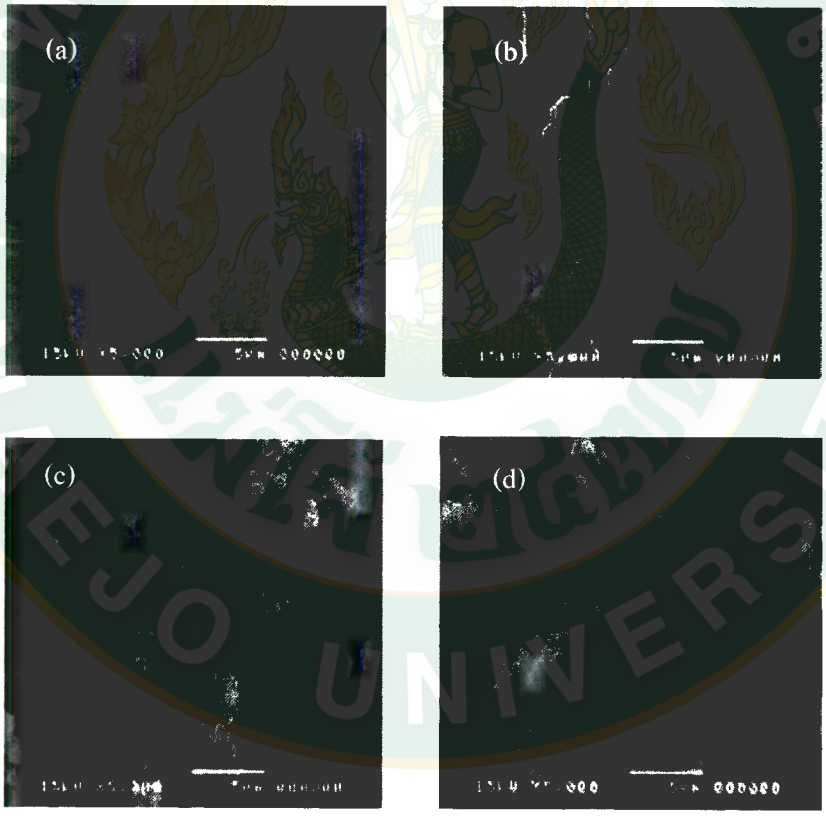


Figure 30 MCs without extract were prepared with different concentrations of PVA solution (a) 0%, (b) 1%, (c) 3% and (d) 5% wt/v

4.2.1 Preparation of MCs containing natural extracts

According to the mechanism proposed by Rochmadi and Hasokawati (Rochmadi and Hasokawati, 2010), two simultaneous steps occurred during the MC formation. The first step was the formation of an emulsion of extract in UF solution. The second step, the pH was adjusted to change the liquid emulsion into an acidic condition. This procedure induced the reaction of urea with formaldehyde at oil-water interface, producing a film of UF polymer as shell material. Resorcinol was used as a cross-linking agent, under acid condition, reacted with formaldehyde generating phenolic resin which was as a part of microcapsule wall (Sun et al., 2013). Ammonium chloride was served as a curing catalyst to help urea and formaldehyde reacted to form a water-insoluble cross-linked polymeric network (Sanchez-Silva et al., 2012).

MC powder containing guava leaf extract is brown and the darker of color powder is attributed to the extract content in MCs (as in **Figure 31**). In addition, the produced MCs containing soap nut extract were various shades of grey depending on the amount of the respective extract (as in **Figure 32**).

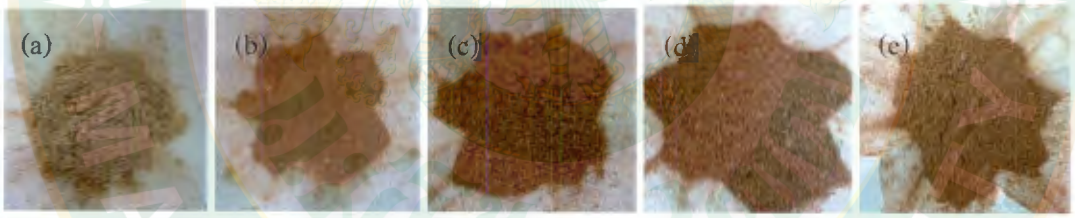


Figure 31 MCs containing guava leaf extract at different weight ratios of extract to urea (a) 1/5, (b) 2/5, (c) 3/5, (d) 4/5 and (e) 5/5

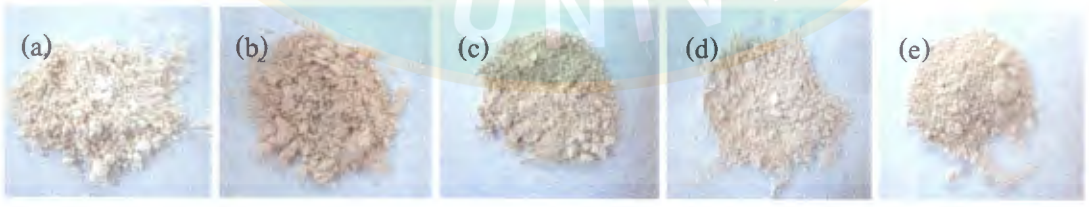


Figure 32 MCs containing soap nut extract at different ratios weight of extract to urea (a) 1/5, (b) 2/5, (c) 3/5, (d) 4/5 and (e) 5/5

4.2.2 Encapsulation yield and encapsulation efficiency

To investigate the amount of extract in the encapsulations various amounts of extract (1, 2, 3, 4 and 5 g) were incorporated in the synthesis of MCs. Increasing the extract amount corresponded to an increasing color intensity of the MCs.

In the preparation of microcapsules, the weight ratio between core to wall (W_{core}/W_{wall}) was varied to be 1/5, 2/5, 3/5, 4/5 and 5/5, respectively in this study. Afterwards, the total amount of phenolic compounds was determined by using Folin-Ciocalteu assay and the results were calculated as the percentage of encapsulation according to **3.3.3**. The result of %EY and %EE as shown in **Table 6** is indicated that %EY was slightly increased by increasing the ratio of core to wall except %EY of at 5/5 was lower. The %EE was found in the range of 79-83%.

Table 6 Encapsulation yield and encapsulation efficiency of MCs containing guava leaf extract

Weight ratio of extract/urea (g/g)	%EY	%EE
0/5	41.10 ± 1.23	-
1/5	34.30 ± 2.36	83.42 ± 4.93
2/5	36.86 ± 3.04	82.13 ± 4.96
3/5	37.91 ± 2.46	84.33 ± 0.98
4/5	40.83 ± 0.77	83.91 ± 3.97
5/5	35.25 ± 1.05	79.46 ± 2.74

For the prepared microcapsules containing soap nut extract the percentage encapsulation yield (%EY) and encapsulation efficiency (%EE) were determined and the results are shown in **Table 7**. For encapsulation yield, the highest %EY was the ratio of extract to urea at 4/5. However, the highest %EE was at 3/5. Actually, the highest %EY should be at 5/5, this result may be due to the size of microcapsules, the small particles was occurred when increasing the ratio of extract to urea as shown in **Figure 36**. Tiny microcapsules probably passed the filter paper so the yield of microcapsules was less than the curtain yield.

Table 7 Encapsulation yield and encapsulation efficiency of MCs containing soap nut extract

Weight Ratio of extract/urea (g/g)	%EY	%EE
1/5	24.37 ± 0.24	27.27 ± 2.12
2/5	26.10 ± 2.48	52.89 ± 5.59
3/5	38.50 ± 2.11	55.76 ± 1.14
4/5	65.89 ± 0.91	59.93 ± 0.45
5/5	59.99 ± 1.59	63.43 ± 1.12

4.3 Characterization of MCs containing extract

After MCs containing of both guava leaf and soap nut extracts were prepared by in situ polymerization, the composition of MCs were analyzed, characterization of surface morphology was conducted and the particle size distribution and thermal characterization were carried out.

4.3.1 The composition of MCs

Figure 33 presents the suggested reaction of PUF and **Figure 34** presents the chemical structure of gallic acid which was represented as the phenolic compounds in guava leaf extract. Phenolic compounds in guava leaf extract consisted of various phenolic compounds such as gallic acid, protocatecuic acid, caffeic acid, ferulic acid, chlorogenic acid, ellagic acid, guavin B, quercetin, leucocyanidin, kaempferol, kaempferol-3-glucoside, guajaverin, avicularin, mecocyanin, quercitrin, β-carotene, lutein and eualyptol, etc. (Gutiérrez et al., 2008). From the **Figure 33**, the main components of PUF were –NH, C=O and –C-N-. Whereas, the main components of phenolic compounds were C=O, C-C and –OH group as shown in **Figure 34**. The structure of PUF MCs was investigated using FTIR to confirm the existing core material with the guava leaf extract. The FTIR spectrum of the guava leaf extract, PUF shell, and PUF MCs containing guava leaf extract are presented in **Figure 35**. The spectra of the PUF shell and PUF MCs containing guava leaf extract show a similar pattern: peaks of C=O stretching vibration at 1650 cm^{-1}, N-H stretching vibration at 1554 cm^{-1}, and C-N stretching vibration at 1245 cm^{-1}. These spectrums confirm the formation of the PUF wall of the MCs. Figure 33 presents the

suggested reaction mechanism of PUF. Furthermore, the presence of bands at 1444 cm^{-1} and 1118 cm^{-1} in the spectrum of PUF MCs containing guava leaf extract correspond to the C-H bending vibration and C-C bending vibration in the spectrum of guava leaf extract. Along with the O-H peak at 3700-3000 cm^{-1}, it shows a broader band which corresponds to the –OH group in phenolic compounds. It shows that the guava leaf extract is successfully encapsulated in the PUF shell.

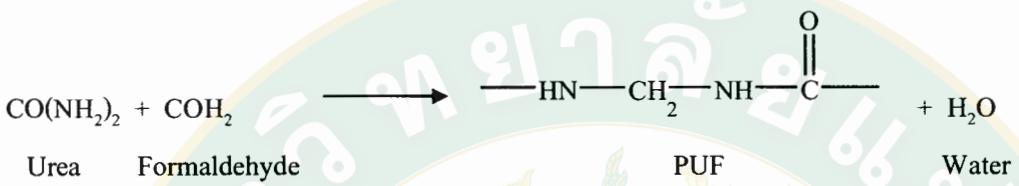


Figure 33 The reaction scheme of formation of PUF

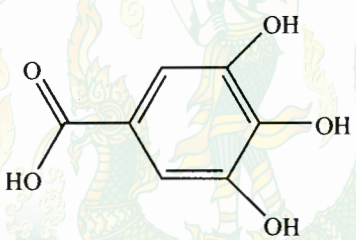


Figure 34 The chemical structure of gallic acid

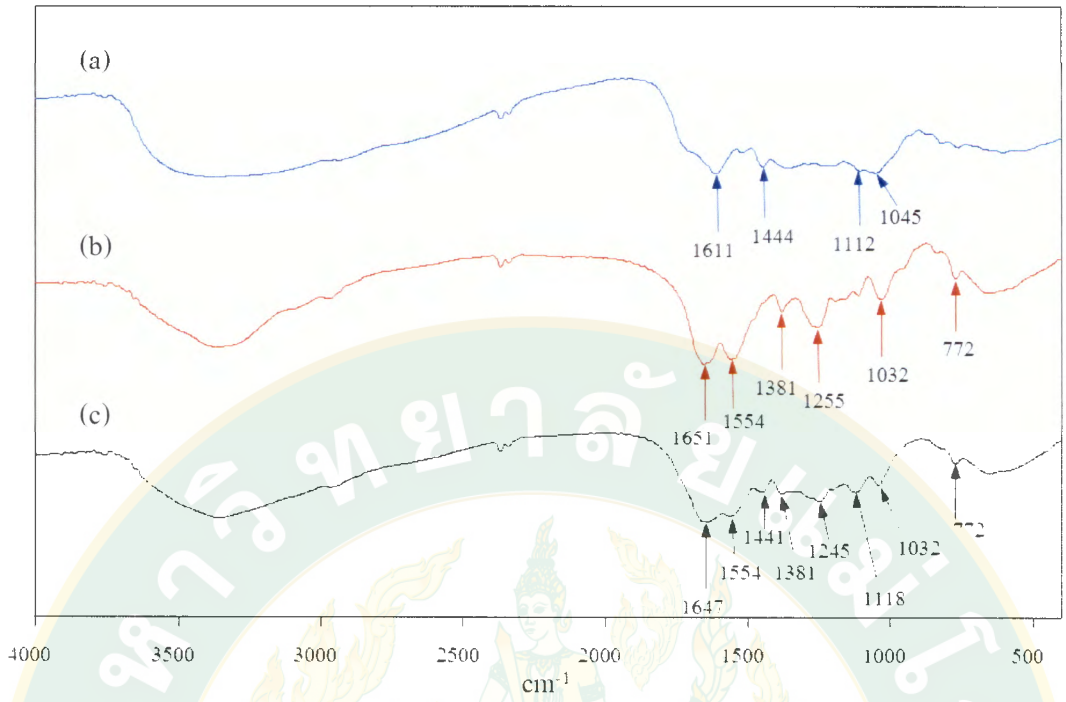


Figure 35 FTIR spectra of (a) guava leaf extract (b) PUF shell (c) PUF MCs containing guava leaf extract

Table 8 Wavenumber (cm^{-1}) and assignment of the FTIR spectra of the mixture of MCs containing guava leaf extract

Approximate assignments	Functional groups
3700 – 3000	O-H stretching
	N-H streching
1650	-NH-CO-NH
1544	-CO-NH-
1444	C-H bending
1118	C-C bending
1032	-C-O-C-

Moreover, the FTIR spectrum of the soap nut extract, PUF shell, and PUF MCs containing soap nut extract are presented in **Figure 36**. The chemical structure of PUF was showed in **Figure 33** and the main components were explained above. Saponins in soap nut extracts were classified as triterpene saponins which were mukurozi-saponin (**Figure 17**), sapindoside B (**Figure 18**) and rarasaponins IV, V and VI (**Figure 19**). From **Figure 17-19**, the main components of saponins were consisted of –OH group and C-O bond. Even though, the band pattern of the PUF shell and PUF MCs spectra was similar, but the absorption bands at 1650 cm^{-1} and 1554 cm^{-1} which corresponded to C=O stretching vibration and N=H stretching vibration in PUF MCs containing soap nut extract were broader than in PUF shell spectra. These caused from their conjugation of C=O groups and NH_2 groups with OH groups in saponins. Along with the O-H peak at 3700-3000 cm^{-1}, it showed a broader band which corresponds to the –OH group in saponin compounds. The result showed that the soap nut extract was successfully encapsulated in the PUF shell.

However, the results from FTIR spectrum did not completely confirm the existance of the extract in PUF MCs. Therefore, additional expcrimented evidence was under taken using scanning electron microscopy (SEM) and thermogravimetric analysis (TGA).

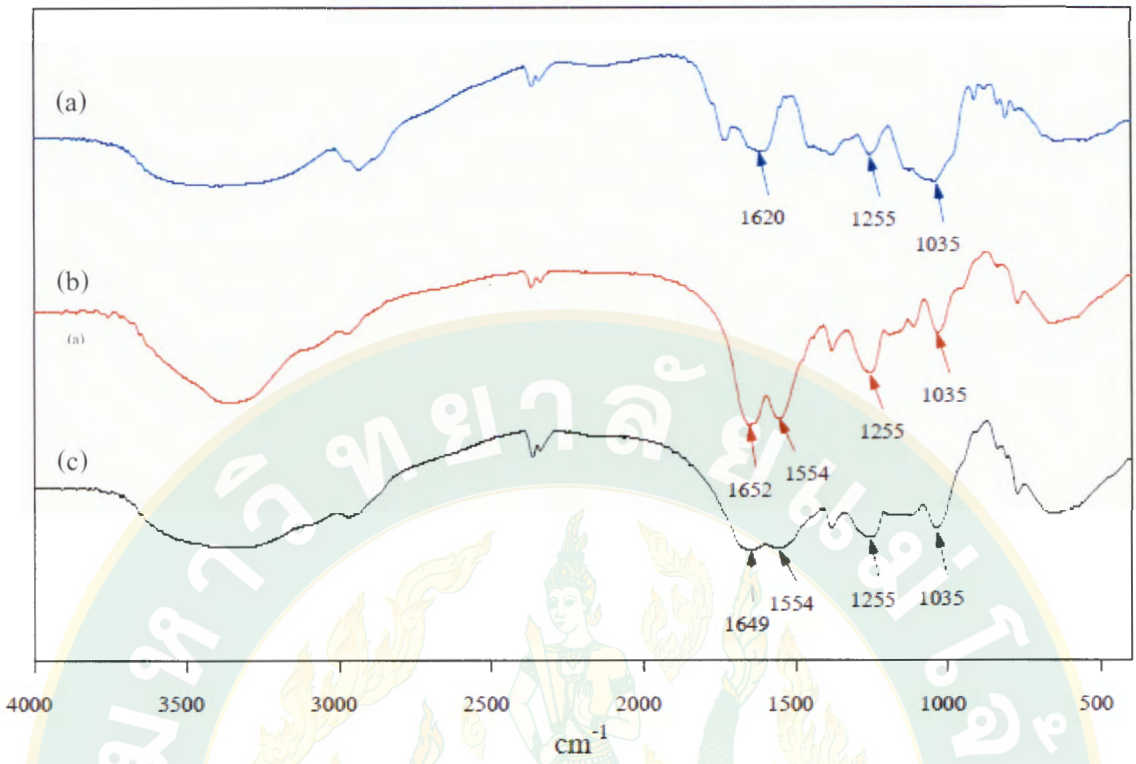


Figure 37 FTIR spectra of (a) soap nut extract (b) PUF shell (c) PUF MCs containing soap nut extract

Table 9 Wavenumber (cm^{-1}) and assignment of the FTIR spectra of the mixture of MCs containing soap nut extract

Approximate assignments	Functional groups
3700 – 3000	O-H stretching N-H streching
1650	-NH-CO-NH
1544	-CO-NH-
1255	-C-O-C-
1035	-C-O-C-

4.3.2 Morphology of MCs

In the preparation of the guava leaf MCs, the weight ratio of extract to urea ($W_{extract}/W_{urea}$) is important to the shape and size of the final MCs. The surface morphologies of obtained microcapsules are illustrated in **Figure 37-39**. MCs without guava leaf extracts show spherical particles in **Figure 37**. When the guava leaf extract is loaded at the ratio of 1/5, MCs exhibit a smaller shape. Besides, compared with **Figure 38b**, the wall materials could not simultaneously deposit onto the drops to form complete sphere. Furthermore, when the ratio of core to wall are increased to 3/5 (**Figure 38c**), the wall materials are not completely converted as the shells and the shape of MCs are irregular. This may be due to the overloading of the core in these particular MCs. These results were similar with the report of Yaun et al. (Yaun et al., 2006), as the UF weight ratio decreased, the surface of MCs gradually got rough. When the weight ratio of UF was lower, the content of free formaldehyde was higher, although the network density of PUF decreased the condensation rate of urea and formaldehyde increased which resulted in quick deposition of PUF nanoparticles on the surface of MCs, forming rougher and more porous outer layer of the PUF shell. Because of the completion of condensation reaction for the higher UF weight ratio resins need longer time compared with the lower UF weight ratio resins, the completion of microencapsulating core with the higher UF weight ratio resins needs more time. As the result, the core material could not encapsulate completely by higher UF weight ratio resins when the other processing parameters were kept constant.

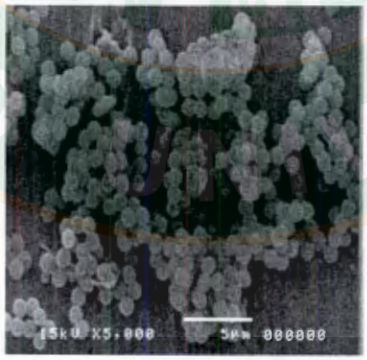


Figure 37 SEM images of PUF MCs without extract

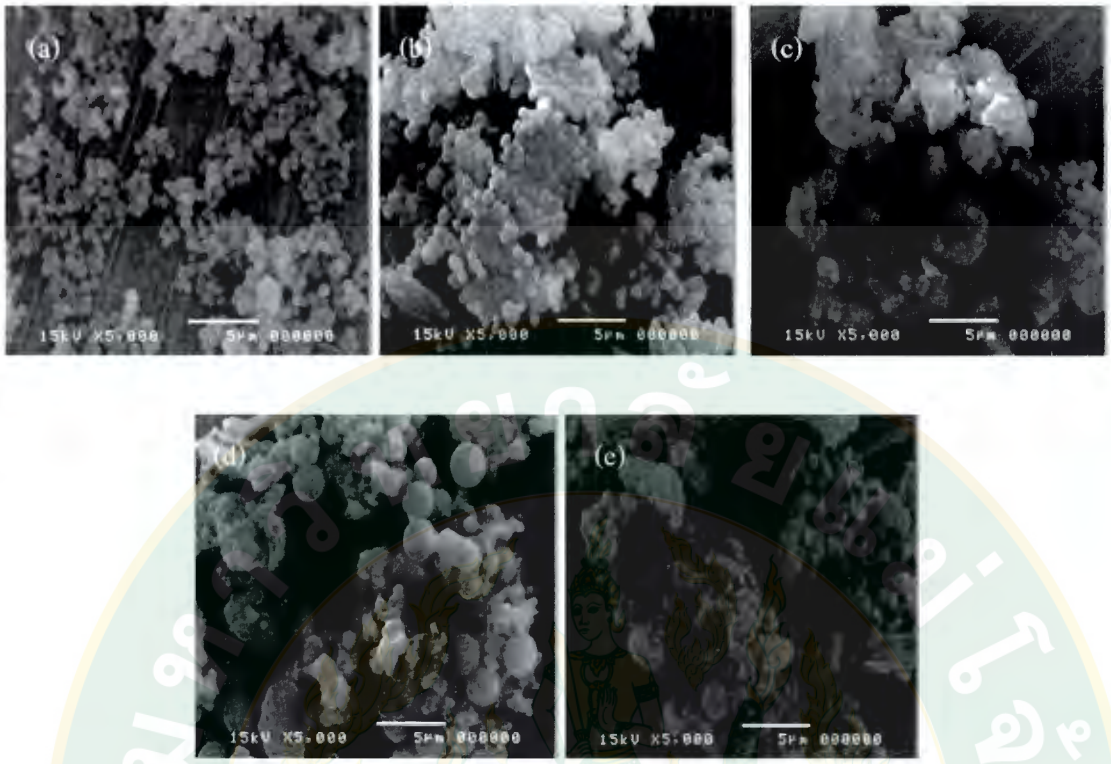


Figure 38 SEM images of PUF MCs containing guava leaf extract at different weight ratios of extract to urea (a) 1/5, (b) 2/5, (c) 3/5, (d) 4/5 and (e) 5/5

For the result of the morphology of MCs containing soap nut extract is illustrated in **Figure 39**. When the soap nut extract was loaded at the ratio of 1/5, MCs exhibited a larger size of particle (**Figure 39a**) than MCs without extract (as in **Figure 37**) which showed spherical particles. Furthermore, when the ratio of core to wall were increased (**Figure 39c-39e**), microcapsules are smaller. This may be affected by surfactant property of saponins.

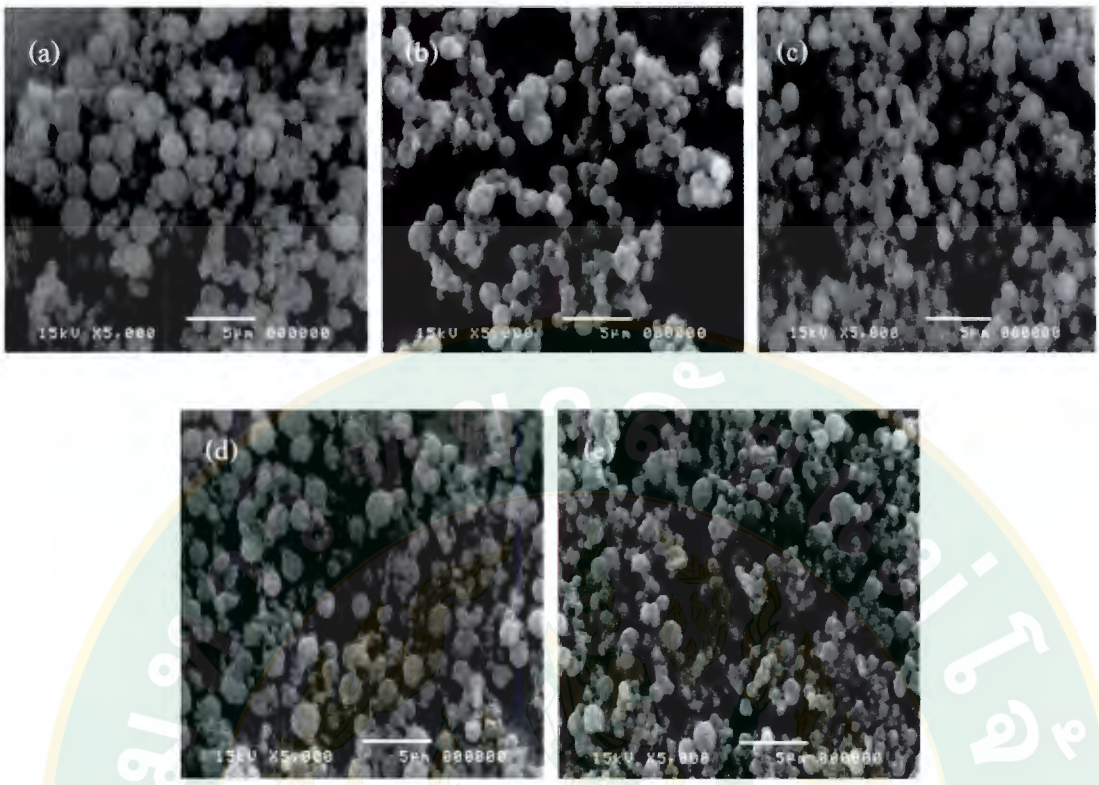


Figure 39 SEM images of PUF MCs containing soap nut extract at different weight ratios of extract to urea (a) 1/5, (b) 2/5, (c) 3/5, (d) 4/5 and (e) 5/5

4.3.3 Particle size

Four MC samples which were MCs without extract (0/5) and MCs containing extract at 1/5, 3/5 and 5/5 weight ratio of extract to urea were selected for particle size analyses. The process was run from room temperature to 500 °C. The results of MC size distribution were described by plotting particle diameter (micrometers) versus the percentage of volume. In **Figure 40** and **Figure 41** the results of MCs containing guava leaf extract and MCs containing soap nut extract, respectively are illustrated. Accordingly in **Figure 40**, the particle diameter of MCs without extract was distributed into 2 ranges: a large group of 8-50 micrometers and a smaller group of 50-140 micrometers. When the guava leaf extract was added at 1/5 weight ratio of extract to urea into MCs, the diameter of MCs was in a wide ranged from 10-150 micrometers and the average diameter was increased. Otherwise the curve of MCs containing guava leaf extract at 3/5 and 5/5 weight ratio was similar and the particle diameter was distributed into 2 groups: a small group was approximately around 10-30 micrometers and a large group was

ranged of 30-250 micrometers. The size of MCs was increased according to the weight of the extract in MCs, thus this may be caused by encapsulation of the extract by PUF. Moreover these results were also corresponded with the result from SEM that MCs was larger when an increasing the guava leaf extract.

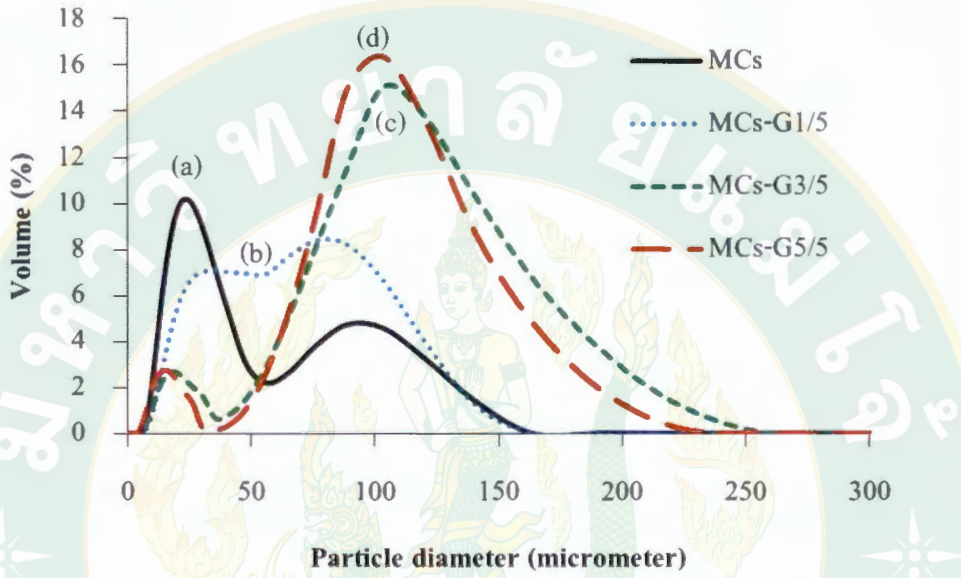


Figure 40 Particle size distribution of MCs containing guava extract at three weight ratios extract to urea (a) — is MCs without extract, (b) ... is MCs containing guava leaf extract at 1/5 weight ratio (c) --- is MCs containing guava leaf extract at 3/5 weight ratio and (d) — - is MCs containing guava leaf extract at 5/5 weight ratio

In **Figure 41** shows the results of the particle size distribution of MCs containing soap nut extract at 1/5 weight ratio of extract to urea ranging from 5-40 micrometers and 50-140 micrometers. After the weight of soap nut extract was increased to 3/5 weight ratio of extract to urea, the particle size of MCs was in a wide range of 10-170 micrometers. MCs containing soap nut extract at weight ratio of 5/5 showed the particle diameter distribution was alike MCs containing soap nut extract at weight ratio of 3/5. At the ratio of 5/5, the particle distribution of MCs became larger due to adhesion of the particles by increasing of saponins which served as surfactant.

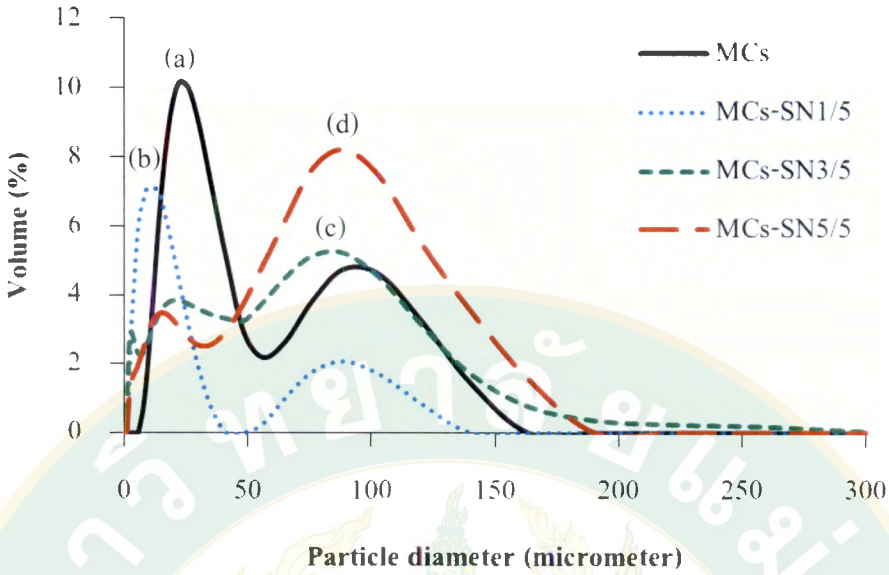


Figure 41 Particle size distribution of MCs containing soap nut extract at three weight ratios extract to urea (a) — is MCs without extract. (b) ... is MCs containing soap nut extract at 1/5 weight ratio (c) --- is MCs containing guava lea soap nut f extract at 3/5 weight ratio and (d) — - is MCs containing soap nut extract at 5/5 weight ratio

The diameters of MCs increased with the increasing of weight ratio of extract to urea (core to wall material). The main reason was that the size of core droplet in emulsion was larger when the weight ratio of core to wall was higher and the other processing parameters were kept constant. Although, the wall thickness may increase owing to the increasing of core material, it slightly affected the diameter of MCs when the core material changed largely. Increasing the core material can form larger size core droplet, and accordingly, the MC size became larger. But excess core materials caused poor dispersion, promoting aggregation of core droplets, resulting in lower yield of MCs at ratio of 5/5. Moreover, the MCs prepared by selecting higher weight ratio of extract to urea were easily fractured due to the thinner wall.

Table 10 Mean diameter of MCs containing guava extract and MCs containing soap nut extract

$Wt_{extract}/Wt_{urea}$	Mean dimeter (μm) of MCs containing	
	Guava leaf extract	Soap nut extract
1/5	48.81 ± 0.06	15.44 ± 0.15
3/5	86.98 ± 0.26	35.41 ± 0.16
5/5	80.76 ± 0.32	47.57 ± 0.27
	Mean dimeter (μm) of MCs without extract	
0/5	36.65 ± 0.06	

4.3.4 Thermal analysis of MCs

Figure 42 showed TGA diagrams of MCs without extract (b), guava leaf extract (b), MCs containing guava leaf extract at 1/5 weight ratio of extract to urea (c), MCs containing guava leaf extract at 3/5 weight ratio of extract to urea (d) and MCs containing guava leaf extract at 5/5 weight ratio of extract to urea (e). Weight loss of guava leaf extract occurred at temperature of 120 °C and did not conclude until 500 °C. TGA curve of all MCs (including without and containing guava leaf extract) showed the same weight loss occurred at temperature of 30-90 °C due to the evaporation of free formaldehyde and water on MCs surface. Following on with a second weight loss occurred at temperature of 220 °C was as a consequence from the decomposition of the PUF shell. Afterward TGA curves of them were different. For the result of MCs without extract the residue of PUF shell was observed the decomposition temperature at approximate 270 °C. The rate of weight loss was quick at around 240-300°C, later the slope was slow down until 500 °C. Comparing the results of TGA diagram of MCs containing guava leaf extract (at 1/5, 3/5 and 5/5 weight ratio of extract to urea) found weight loss temperature of the MCs containing guava leaf extract at 1/5, 3/5 and 5/5 occurred approximate at 260 °C, 250 °C and 235 °C, respectively, because guava leaf extract was decomposed. The weight loss temperature of MCs containing guava leaf extract with three ratios was decreased with increasing the content of extract or decreasing the content of UF shell in MCs. It was indicated that the higher weight ratio of extract was encapsulated in MCs, the shell of MCs was thinner. Therefore, the weight

loss temperature of MCs containing guava leaf extract was lower according to the weight ratio of extract to urea.

For the result of guava leaf extract, it can explain by according the report of Philpot and Mutch (Philpot and Mutch, 1969). They revealed that the major components of natural fuel were cellulose, usually amounting to 40-45% by weight, lignin 30% and hemicellulose 25%. Lignin did not undergo pyrolysis below 400 °C. Therefore, TGA curve of guava leaf extract showed the slope undergone until 500 °C.

TGA curves for the soap nut extract (a) are shown below in **Figure 43** with MCs without extract (b), MCs containing soap nut extract at 1/5 weight ratio of extract to urea (c), MCs containing soap nut extract at 3/5 weight ratio of extract to urea (d) and MCs containing soap nut extract at 5/5 weight ratio of extract to urea (e). The mass loss of soap nut extract occurred approximately at 210 °C and the analysis was performed to 500 °C. For the TGA curves of MCs containing soap nut extract and containing guava leaf extract (three weight ratios of extract to urea: 1/5, 3/5 and 5/5), these exhibited similar thermal behavior in range of the temperature at 220-320 °C. Weight loss temperature of the MCs containing guava leaf extract at 1/5, 3/5 and 5/5 occurred approximate at 260 °C, 250 °C and 275 °C, respectively. Although, the weight loss temperature of 5/5 ratio was higher than of 3/5 ratio, this may be caused the adhesion of the MC particles became the larger shape as explained in **4.3.3**. The MCs were attached to form the large particles so they were decomposed at higher temperature.

Moreover, the slope quickly decreased whereby the weight loss percentage of MCs containing soap nut extract at 1/5 and 3/5 weight ratio extract to urea was about 40%. However, the weight loss percentage of MCs containing soap nut extract at 5/5 was higher at about 60%. These results indicate that the MCs containing soap nut extract at 5/5 showed better the encapsulation of soap nut extract than the MCs containing soap nut extract at 1/5 and 3/5.

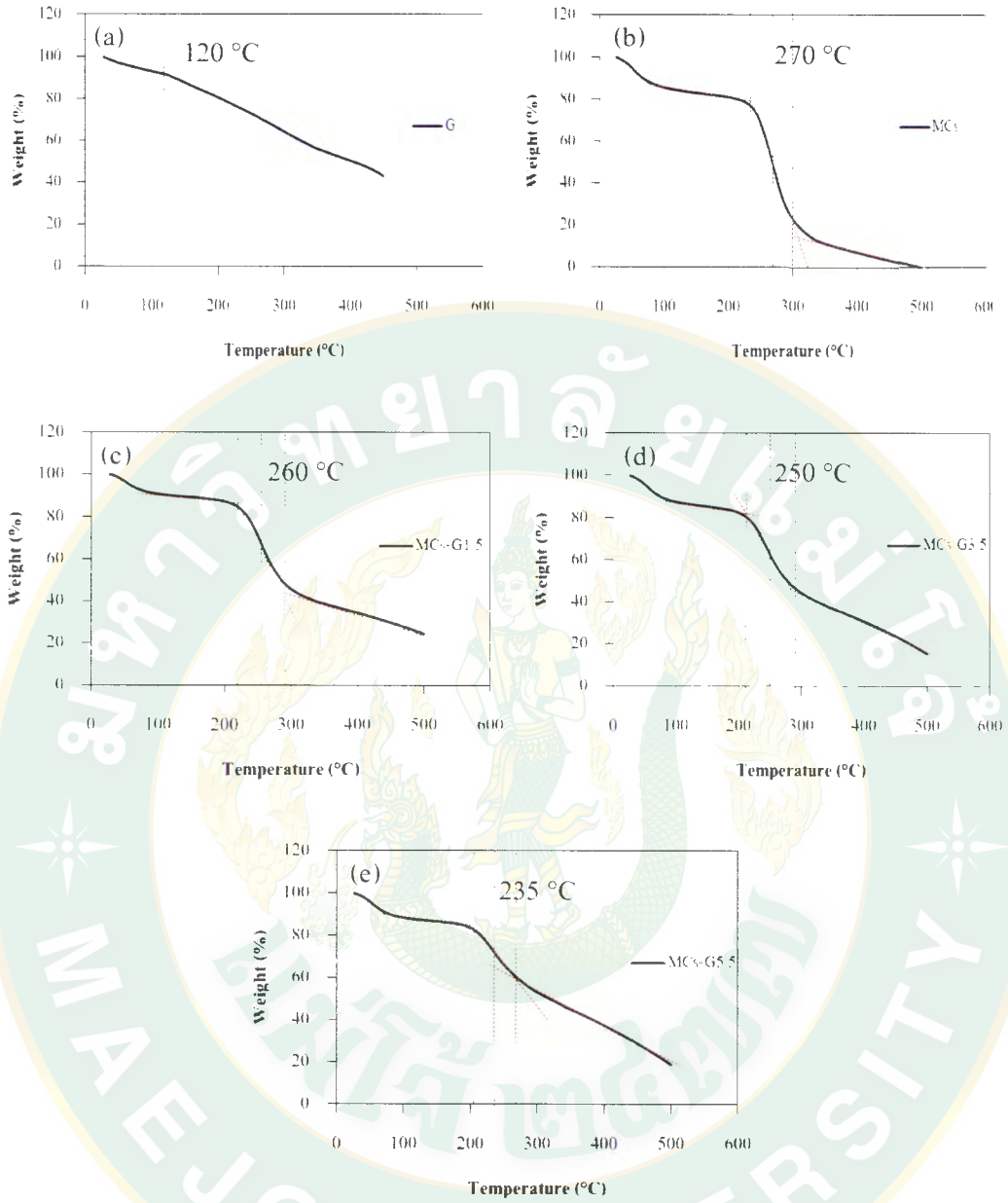


Figure 42 TGA diagrams of (a) guava leaf extract, (b) MCs without extract, (c) MCs containing guava leaf extract at 1/5 weight ratio of extract to urea, (d) MCs containing guava leaf extract at 3/5 weight ratio of extract to urea and (e) MCs containing guava leaf extract at 5/5 weight ratio of extract to urea

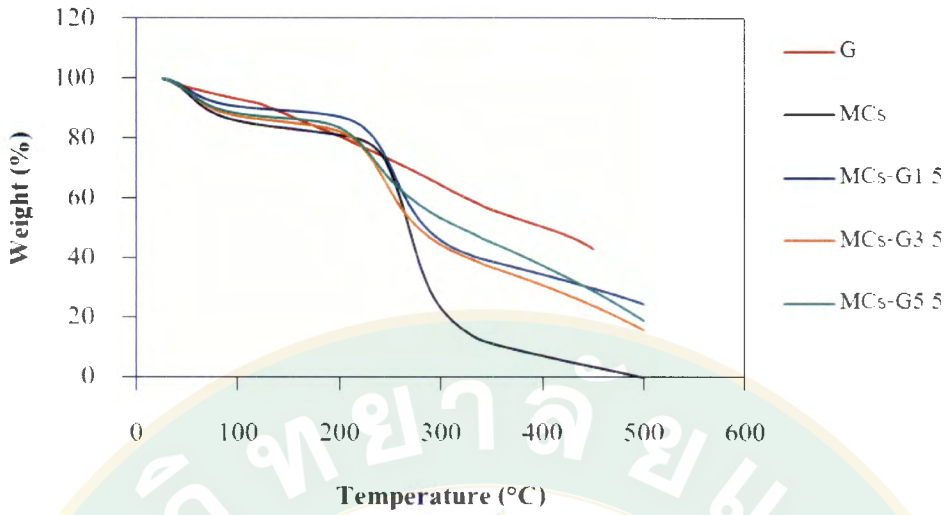


Figure 42 TGA diagrams of guava leaf extract (red line), MCs without extract (black line), MCs containing guava leaf extract at 1/5 weight ratio of extract to urea (blue line), MCs containing guava leaf extract at 3/5 weight ratio of extract to urea (yellow line) and MCs containing guava leaf extract at 5/5 weight ratio of extract to urea (green line) (cont.)

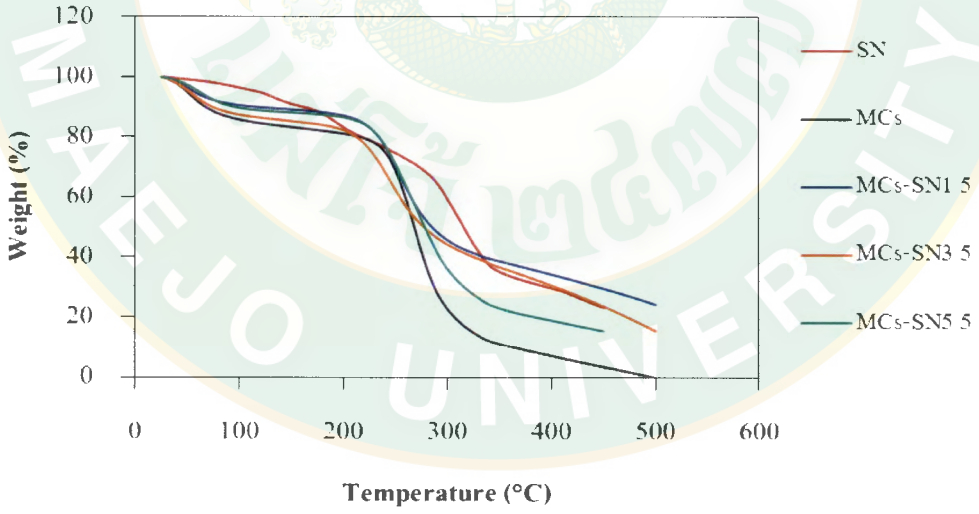


Figure 43 TGA diagrams of soap nut extract (red line), MCs without extract (black line), MCs containing soap nut extract at 1/5 weight ratio of extract to urea (blue line), MCs containing soap nut extract at 3/5 weight ratio of extract to urea (yellow line) and MCs containing soap nut extract at 5/5 weight ratio of extract to urea (green line)

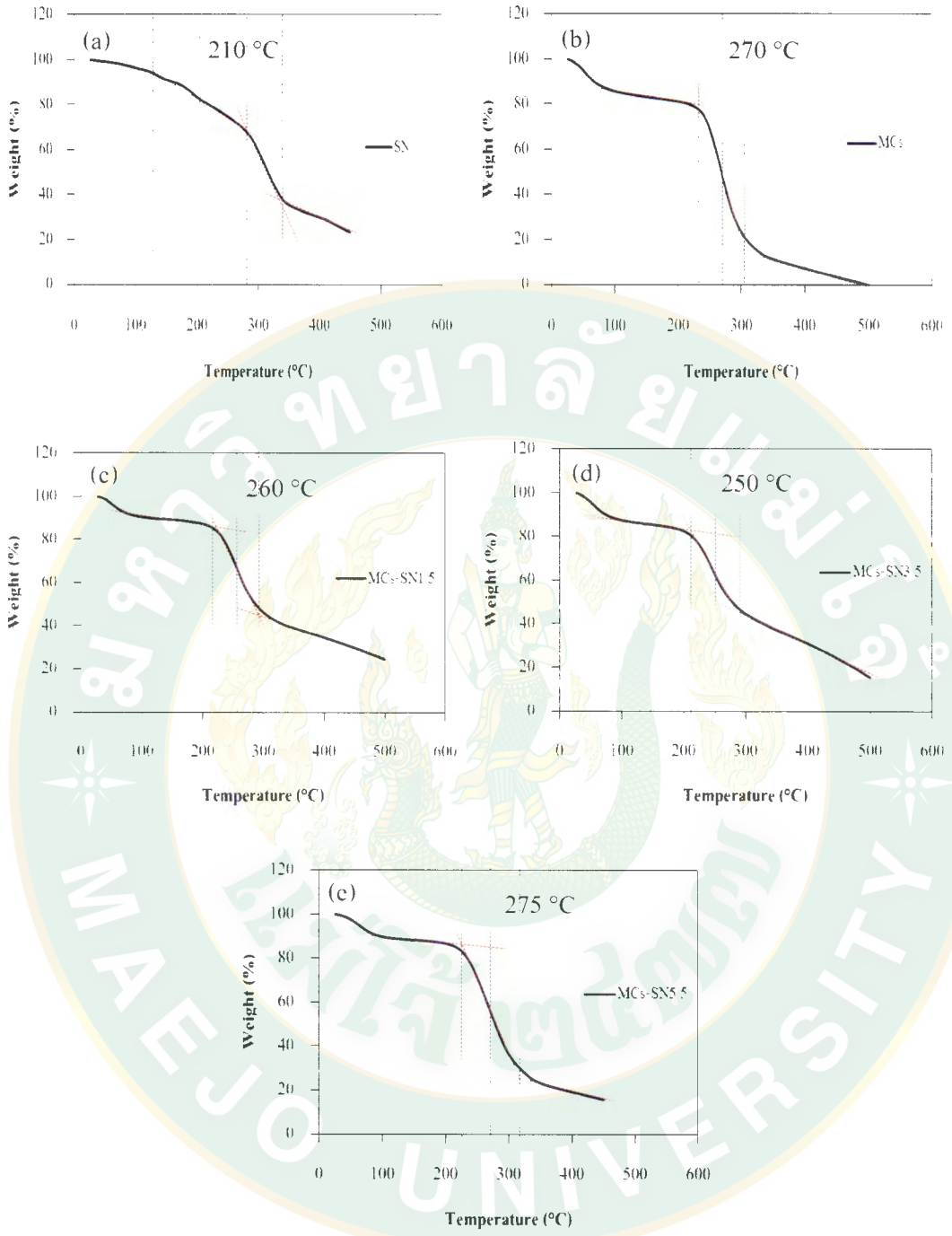


Figure 43 TGA diagrams of soap nut extract (a), MCs without extract (b), MCs containing soap nut extract at 1/5 weight ratio of extract to urea (c), MCs containing soap nut extract at 3/5 weight ratio of extract to urea (d) and MCs containing soap nut extract at 5/5 weight ratio of extract to urea (e) (cont.)

4.4 Characterization of finished fabric

Cotton fabric was finished by screen printing method (as shown in **Figure 44)** with antibacterial agents, guava leaf extract, MCs containing guava leaf extract, soap nut extract and MCs containing soap nut extract. MCs containing extract at 5/5 weight ratio of extract to urea was selected to use as an antibacterial agent. The concentration of all the antibacterial agents was used at 50 g/l. The antibacterial agents were deposited on the fabric surface with the assistance of helizarin, fixapret ECO, condensol and zinc nitrate hexahydrate. Therefore, helizalin, fixapret ECO, condensol and zinc nitrate hexahydrate were responsible as binder, fixing agent and catalyst for condensol ECO, respectively. After cotton fabrics finished with antibacterial agents, they were dried at 105 °C to eliminate water and cured at 170 °C to fix the antibacterial agent on surface of fabrics.

After the cotton fabrics were finished with the antibacterial agents, they were examined by both optical microscope and SEM for color surface and surface morphology. The final color of finished cotton fabrics became the same color as the extract or MCs after treatment with extract or the MCs. The cotton fabric finished with guava leaf extract has a more intense homogeneous brown color and a smoother surface than the cotton fabric finished with MCs containing guava leaf extract (**Figure 45** and **Figure 46)**. While the cotton fabric finished with MCs containing guava leaf extract showed homogenous brown spot. As well as the cotton fabrics finished with soap nut extract and MCs containing soap nut extract were homogenous light yellow and homogenous light brown spot (**Figure 47** and **Figure 48**). After the fabric samples were examined by SEM, it was concluded that the attached spots on cotton fabric surface were MCs.

Figure 44 Optical microscope and SEM images of cotton fabrics

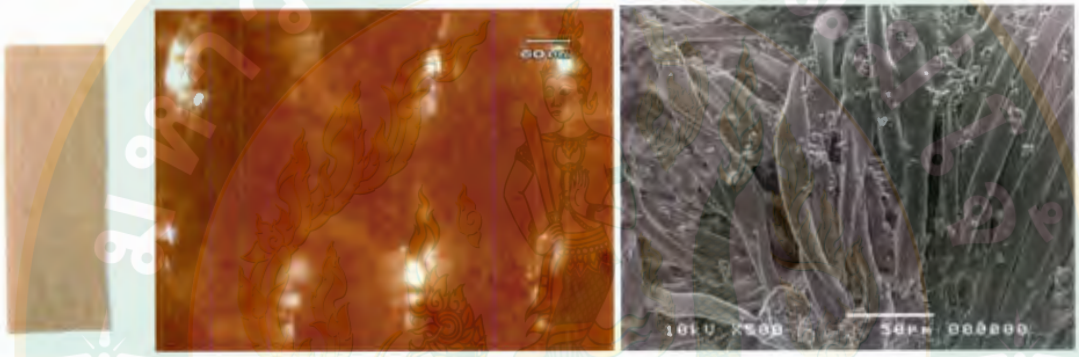


Figure 45 Optical microscope and SEM images of cotton fabrics finished with guava leaf extract

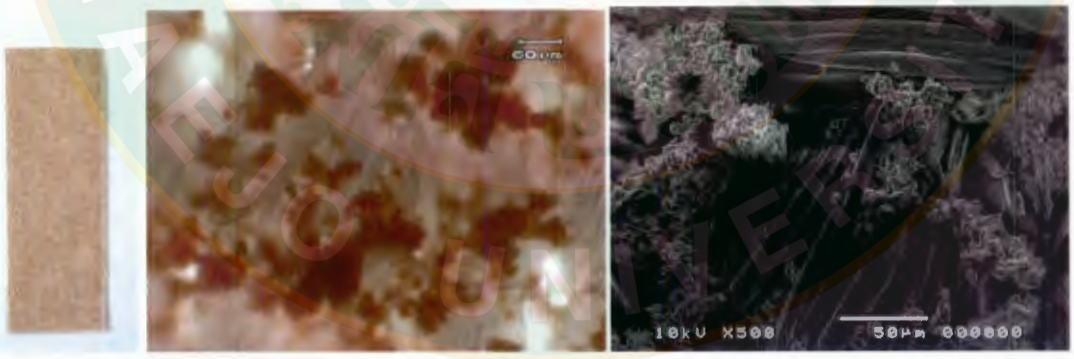


Figure 46 Optical microscope and SEM images of cotton fabrics finished with MCs containing guava leaf extract at 5/5 weight ratio of extract to urea

Figure 47 Optical microscope and SEM images of cotton fabrics finished with soap nut extract

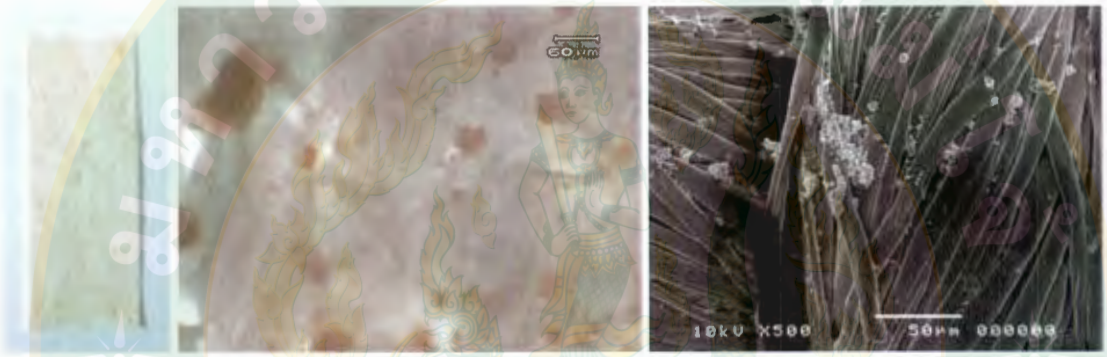


Figure 48 Optical microscope and SEM images of cotton finished with MCs containing soap nut extract at 5/5 weight ratio of extract to urea

4.5 Testing of finished fabric

4.5.1 Antibacterial activity

In order to assess the antibacterial activity for finished cotton fabrics, the testing was carried out according to the standard methods such as disc diffusion method and also confirmed with parallel streak method. The experiments were performed taking gram negative *E.coli* and gram positive *S.aureus* as model bacteria. Test specimens in this study were the unfinished cotton fabric (control fabric), finished cotton fabric with guava leaf extract, finished cotton fabric with MCs containing guava leaf extract, finished cotton fabric with soap nut extract and finished cotton fabric with MCs containing soap nut extract.

Disc diffusion method (SN 19025-1998)

First, the finished fabrics were screened their antibacterial activity against *E.coli* and *S.aureus* using disc diffusion method and the inhibition zone was observed. The fabrics samples were cut into a circle (∅=20 mm) and sterilized by UV-visible electromagnetic radiation. Three concentrations (10^8, 10^7 and 10^6 CFU/ml) of the test organisms, G(-) *E.coli* and G(+) *S.aureus*, were used for the swab by a sterilized cotton bud over the surface of nutrient agar plates. After incubation at 37°C for 24 hr, the inhibition zone was observed. If the fabric samples expressed the antibacterial activity, the clear zone was obtained. The results of the screening test of the fabrics finished with guava leaf extract and MCs containing guava leaf extract against *E.coli* and *S.aureus* are presented in **Figure 49** and **Figure 50**, respectively. Moreover, the antibacterial activity is summarized in **Table 8**. From **Figure 49**, it is shown there is no zone of inhibition of all finished fabrics at all concentrations of *E.coli*, 10^8, 10^7 and 10^6 CFU/ml. It was obvious that fabric finished with guava leaf extract and fabric finished with MCs containing guava leaf extract could not inhibit the growth of *E.coli*. Nevertheless, the inhibition zone could be seen in fabrics finished with guava leaf extract and fabrics finished with MCs containing guava leaf extract against *S.aureus* **(Figure 50)**, it depicted that they could inhibit the growth of *S.aureus*.

(a) (b) (c)

Figure 49 Antibacterial activity of fabric finished with guava leaf extract and cotton fabric finished with MCs containing guava leaf extract by disc diffusion method against *E.coli* (a) 10^8 CFU/ml (b) 10^7 CFU/ml (c) 10^6 CFU/ml when (1); unfinished cotton fabric (2); cotton fabric finished with guava leaf extract and (3); cotton fabric finished with MCs containing guava leaf extract

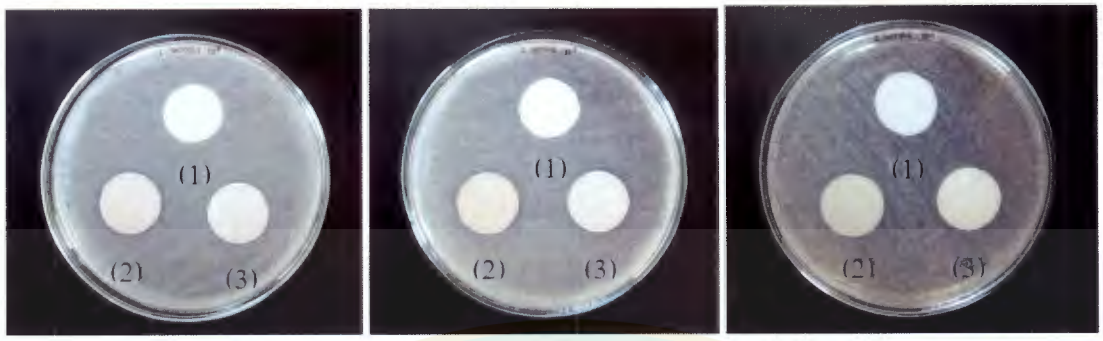
(a) (b) (c)

Figure 50 Antibacterial activity of fabric finished with guava leaf extract and cotton fabric finished with MCs containing guava leaf extract by disc diffusion method against *S.aureus* (a) 10^8 CFU/ml (b) 10^7 CFU/ml (c) 10^6 CFU/ml when (1); unfinished cotton fabric (2); cotton fabric finished with guava leaf extract and (3); cotton fabric finished with MCs containing guava leaf extract

Also in addition, the fabric finished with soap nut extract and MCs containing soap nut extract did not show the inhibition zone against *E.coli*, but it was affected to *S.aureus* (**Figure 51** and **Figure 52**). From the results it was concluded that fabric finished with all antibacterial agents exhibited antibacterial activity against *S.aureus* based on zone of inhibition. The observed inhibition zone is shown in **Table 11**.

(a) (b) (c)

Figure 51 Antibacterial activity of fabric finished with soap nut extract and cotton fabric finished with MCs containing soap nut extract by disc method against *E.coli* (a) 10^8 CFU/ml (b) 10^7 CFU/ml (c) 10^6 CFU/ml when (1); unfinished cotton fabric (2); cotton fabric finished with soap nut extract and (3); cotton fabric finished with MCs containing soap nut extract



(a) (b) (c)

Figure 52 Antibacterial activity of fabric finished with soap nut extract and cotton fabric finished with MCs containing soap nut extract by disc diffusion method against *S.aureus* (a) 10^8 CFU/ml (b) 10^7 CFU/ml (c) 10^6 CFU/ml when (1); unfinished cotton fabric (2); cotton fabric finished with soap nut extract and (3); cotton fabric finished with MCs containing soap nut extract

Table 11 Antibacterial activity of finished cotton fabrics against *E.coli* and *S.aureus*

Fabric samples	Antibacterial activity					
	***E. coli* (CFU/ml)**			***S. aureus* (CFU/ml)**		
	10^8	**10^7**	**10^6**	**10^8**	**10^7**	**10^6**
Unfinished cotton fabric	-	-	-	-	-	-
Cotton fabric finished with guava leaf extract	-	-	-	+	+	+
Cotton fabric finished with MCs containing guava leaf extract	-	-	-	+	+	+
Cotton fabric finished with soap nut extract	-	-	-	+	+	++
Cotton fabric finished with MCs containing soap nut extract	-	-	-	+	+	++

- = No activity

\+ = Weak antibacterial activity, mean inhibition zone lower 5 mm

++ = Moderate antibacterial activity, mean inhibition zone range 5-10 mm

Parallel steak method (AATCC 147-2004)

To confirm the efficiency of antibacterial activity against *S.aureus*. the finished fabrics were assessed for antibacterial activity against *E. coli* and *S. aureus* using AATCC147-2004 method with modification. In this test. fabrics samples were in size of 10x50 mm and followed with sterilization by UV-visible electromagnetic radiation. 10^{6} CFU/ml of test organisms were prepared by adjusting the bacterial suspension turbidity to a McFarland Standard NO. 0.5. Next. bacteria was streaked 5 lines on the surface of nutrient agar plate without refilling the loop. After the fabrics samples were placed on the agar plate. they were incubated at 37°C for 24 hr. The results are shown in **Figure 53 - 56**. **Figure 53** and **Figure 55** show there was no zone of inhibition in any of the fabrics against *E. coli*. The unfinished cotton fabric was shown by **Figure 53(b)** and **Figure 55(b)**. cotton fabric finished with guava leaf extract soap nut extract was **Figure 53(a)** and **Figure 55(a)**. and cotton fabric finished with MCs containing guava leaf extract MCs containing soap nut extract was **Figure 53(c)** and **Figure 55(c)**. However. there was no growth of *E. coli* underneath the fabric finished with guava leaf extract soap nut extract and the fabric finished with MCs containing guava leaf extract MCs containing soap nut extract.

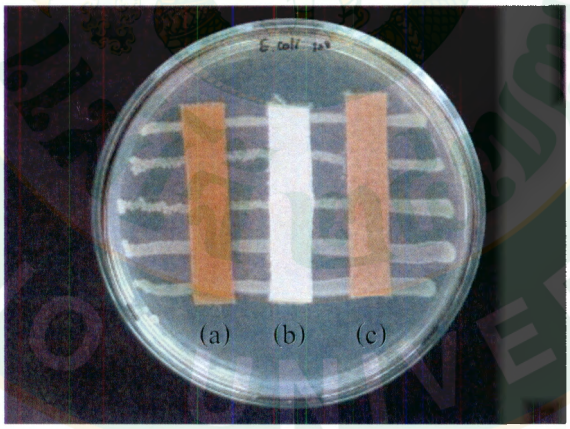


Figure 53 Antibacterial activity by AATCC 147 standard against *E.coli* of (a) cotton fabric finished with guava leaf extract (b) unfinished cotton fabric (c) cotton fabric finished with MCs containing guava leaf extract

Figure 54 shows the result of antibacterial activity against *S. aureus*. The fabric finished with MCs containing guava leaf extract had an obviously larger zone of inhibition that of

the fabric finished with guava leaf extract **Figure 54(a)**. The results showed that fabric finished with MCs containing guava leaf extract showed good antibacterial activity against *S. aureus*. Although the fabric finished with guava leaf extract had no inhibition zone. *S. aureus* could not grow underneath the fabric. This result indicated that cotton fabric finished with MCs containing guava leaf extract could inhibit *S. aureus* better than *E. coli*.

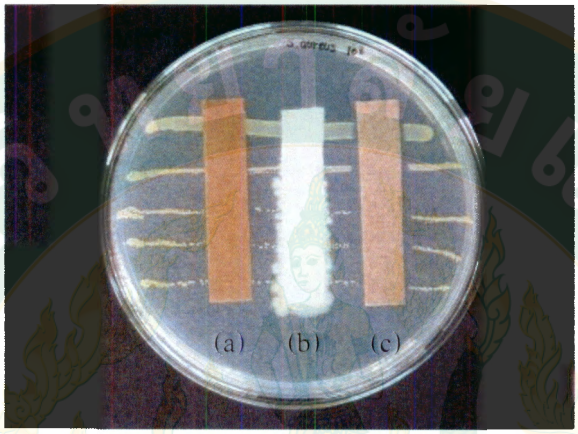


Figure 54 Antibacterial activity results against *S.aureus* using AATCC 147 of (a) cotton fabric finished with guava leaf extract (b) unfinished cotton fabric (c) cotton fabric finished with MCs containing guava leaf extract

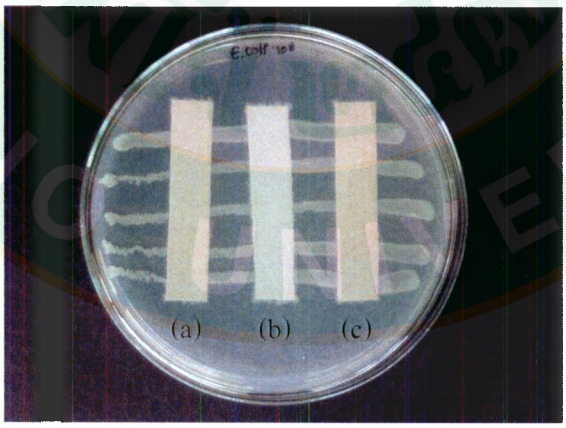


Figure 55 Antibacterial activity results against *E.coli* using AATCC 147 of (a) cotton fabric finished with soap nut extract (b) unfinished cotton fabric (c) cotton fabric finished with MCs containing soap nut extract

Moreover, the fabrics finished with soap nut extract and MCs containing soap nut extract gave good antibacterial activity against *S.aureus*. The obtained inhibition zone of both fabrics is shown in **Figure 56**. Comparing these fabrics with unfinished cotton fabric it was found *S.aureus* could grow underneath the fabric. The summery of the results for antibacterial assessment by AATCC 147 standard is shown in **Table 12** suggested that all of the fabrics finished with the antibacterial agents, guava leaf extract, MCs containing guava leaf extract, soap nut extract and MCs containing soap nut extract was considered efficient for the antibacterial activity against *S.aureus*, but could not inhibit the growth of *E.coli* in the present work.

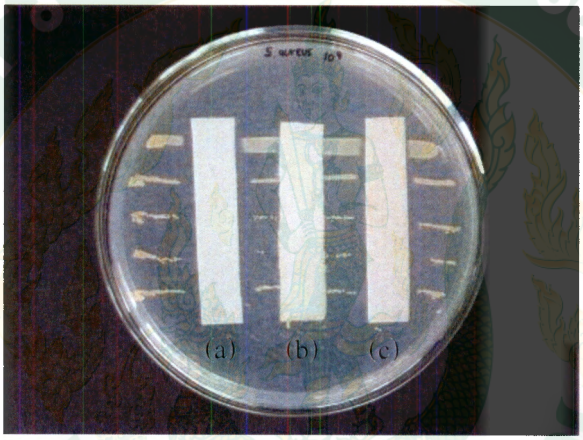


Figure 56 Antibacterial activity results against *S.aureus* using AATCC 147 of (a) cotton fabric finished with soap nut extract (b) unfinished cotton fabric (c) cotton fabric finished with MCs containing soap nut extract

The difference in antibacterial activity between gram positive (*E.coli*) and gram negative (*S.aureus*) bacteria was attributed to the structure of their perspective walls. The cell wall of gram negative bacteria is comprised of a thin peptidoglycan layer and lipopolysaccharide layer. The lipopolysaccharides are composed of covalently linked lipids and polysaccharides with a negative charge but provide a weak permeability barrier to the agent with a positive charge. Whereas, the cell wall of gram positive bacteria was composed of a thick peptidoglycans layer consisting of linear polysaccharide chains cross-linked by short peptides to form a three dimensional rigid structure. This rigid layer makes it difficult for the agent with a positive charge to attach to penetrate through cell wall (Tamboli and Lee, 2013).

The resistance of the gram negative bacteria could be attributed to its cell wall structure. Gram negative bacteria has thick layer of phospholipids slightly than the peptidoglycan comparing to the gram positive which has thick layer of peptidoglycan. The negative charges of the phospholipids improve the adhesion of poly cationic polymer on the polymer on the cell wall (Moussa et al., 2011). Gram negative bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding the plant extract. In addition, it has been reported earlier that gram negative bacteria were usually more resistant to the plant-origin antimicrobials and even showed no effect, compared to gram positive bacteria. Gram positive bacteria had a mesh like peptidoglycan layer which was more accessible to permeation by the extracts (Biswas et al., 2013).

The mechanism of antibacterial action of phenolic compounds and saponins can be described by Stefanović et al. Phenolic compounds (mainly compound in guava leaf extract) were consisted of a hydroxyl functional group (-OH) which was a negative charge. The site(s) and number of hydroxyl groups on the phenol group were considered to be related to their relative toxicity to bacteria, with evidence that increased hydroxylation results in increased toxicity. And the mechanism of terpenes was speculated to involve membrane disruption by the lipophilic compounds. Whereby, the structure of saponins in soap nut was classified to be terpenoid sapogenin. Moreover, Pasaribu et al. (Pasaribu et al., 2014) were reported that due to the ability of saponins to reduce the tension of solution so saponins were caused to combine of the nonpolar sapogenin and the water soluble side chain. Saponins can directly bind with phospholipids of gram positive bacteria cell wall, hence, lysis the cell wall of bacteria.

Table 12 Antibacterial assessment of the finished cotton fabrics against *E.coli* and *S.aureus* by AATCC 147-2004

Fabric samples	Mean inhibition zone (mm)	
	Escherichia coli	*Staphylococcus aureus*
Unfinished cotton fabric	0	0
Cotton fabric finished with guava leaf extract	0	11.7 ± 0.1
Cotton fabric finished with MCs containing guava leaf extract	0	12.9 ± 1.2
Cotton fabric finished with soap nut extract	0	16.0 ± 1.5
Cotton fabric finished with MCs containing soap nut extract	0	14.0 ± 2.8

4.5.2 Wash durability

The fabrics finished with the antibacterial agents were further tested for wash durability according ISO 105-C01. Durability of the finished fabrics due to washing was examined for up to 5 washing cycles in aqueous solution containing 5 g/l standard surfactant at 40 °C for 30 min for each cycle. Finally, the antibacterial activity was demonstrated as inhibition zone around the fabrics according to AATCC 147-2004 standard with modification. The results of antibacterial testing of finished fabrics after washing 1 and 5 cycles are shown in **Figure 57** and **Figure 58,** respectively. **Figure 57(a)** and **Figure 57(b)** show there was no zone of inhibition around the fabrics finished with guava leaf extract, MCs containing guava leaf extract, soap nut extract and MCs containing soap nut extract so they could not inhibit the growth of *S.aureus*. These results indicated all finished fabrics showed loss of the antibacterial activity against *S.aureus* after washing 1 cycle. However, *S.aureus* could not growth underneath all the finished fabrics.

Figure 58 illustrate wash durability of the fabrics finished with guava leaf extract, MCs containing guava leaf extract, soap nut extract and MCs containing soap nut extract after washing 5 cycles. After the fabrics finished with guava leaf extract, MCs containing guava

leaf extract, soap nut extract and MCs containing soap nut extract were washed 5 cycles, they were assessed the antibacterial activity. The results showed poor wash durability against *S.aureus* due to the finished fabrics did not observed the inhibition zone around the finished fabrics (**Figure 58(a)** and **Figure 58(b)**) but *S.aureus* could not growth underneath the finished fabrics. It was seen that no the inhibition zone could be obtained around the finished fabrics after washing for 1 and 5 cycles. Thus, it was indicated that the both of the direct treatment and microencapsulation method treatment on the cotton surface was easily removed by washing.

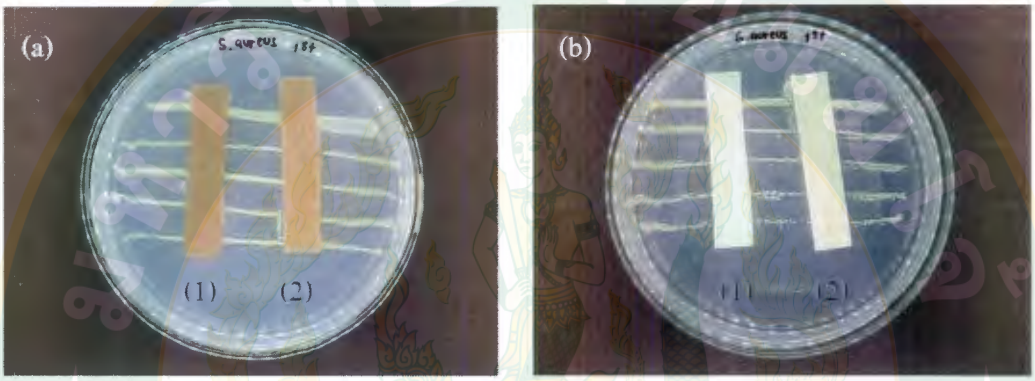


Figure 57 Wash durability after 1 cycle (a) 1; fabric finished with guava leaf extract and 2; MCs containing guava leaf extract and (b) 1; fabric finished with soap nut extract and 2; MCs containing soap nut extract

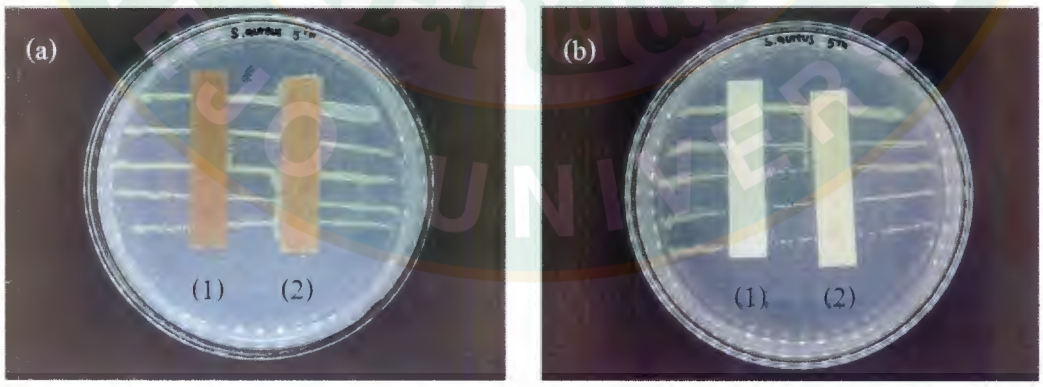


Figure 58 Wash durability after 5 cycles (a) 1; fabric finished with guava leaf extract and 2; MCs containing guava leaf extract and (b) 1; fabric finished with soap nut extract and 2; MCs containing soap nut extract

4.5.3 Determination of free formaldehyde amount

Both the fabric finished with extract and the fabric finished with MCs were analyzed for formaldehyde residue according to the BS EN ISO 14184-1:1999 standard. This experiment was performed to the finished fabrics that may contain formaldehyde which was used as the shell material of MCs. It provided to confirm the antibacterial activity of the finished fabrics. Since formaldehyde has been a common antiseptic or bacteriostatic agent (Neely, 1962) so it can inhibit or kill microorganisms. Moreover, formaldehyde caused respiratory inflammation by inhalation and skin inflammation by skin contact but also irritated the eyes, moreover, formaldehyde may cause allergies and cancer to consumer.

The detection of formaldehyde was based on the reaction of formaldehyde with acetyl acetone (2,4-pentadione) and ammonia. This reaction was produced 3, 5-diaacetly-1,4-dihydrolutidine (DDL), which was absorbed the absorbance at 410 nm and 510 nm. The detection limit of BS EN ISO 14184-1:1999 part I was 20 mg/kg.

The results showed that the free formaldehyde amount on the fabric finished with guava leaf extract and MCs containing guava leaf extract were 0.30 and 1.51 ppm/g of fabric. Whereas, the fabrics finished with soap nut extract and MCs containing soap nut extract containing formaldehyde was approximately 0.32 and 0.69 ppm/g of fabric. As the weight of each sample fabric for antibacterial testing was approximately 0.4 g, it can be assumed that the fabric samples contained 0.12, 0.60, 0.13 and 0.28 ppm formaldehyde for the cotton fabric finished with guava leaf extract, the cotton fabric finished with MCs containing guava leaf extract, the cotton fabric finished with soap nut extract and the cotton fabric finished with MCs containing soap nut extract, respectively. Thus, it is likely that the amount of formaldehyde did not affect the inhibition of organism growth.

Table 13 Formaldehyde amount on finished fabrics

Fabric samples	Formaldehyde amount (ppm/g of fabric)	Formaldehyde amount on 10x50 mm of fabric (ppm)
Fabric finished with guava leaf extract	0.30	0.12
Fabric finished with MCs containing guava leaf extract	1.51	0.60
Fabric finished with soap nut extract	0.32	0.13
Fabric finished with MCs containing soap nut extract	0.69	0.28

Above data (**Table 13**) was lower than the detection limit (20 mg/kg or 20 μg/g) and this test result shall be reported as not-detectable.

CHAPTER 5

CONCLUSIONS

The extracts of guava leaf and soap nut pericarps were used as the antibacterial agent from natural products. Guava leaf and soap nut pericarps were extracted by hot water extraction. Total phenolic contents in guava leaf extract were 28.1 mg GAE/g and 38.8 mg TAE/g of guava leaf extract. And total saponins in soap nut extract were 246.0 mg/g extract. After that, the both of the extracts from guava leaf and soap nut were screened for antibacterial activity by agar diffusion test. The results were found they can inhibit the growth of *S.aureus* and *E.coli.*

Microencapsulation of the natural extracts was used in this study for the protection and long acting release of the antibacterial agents. MCs containing the extracts were prepared by *in situ* polymerization using urea and formaldehyde as a wall materials. A 5% PVA solution was used in the reaction due to it gave the smallest MC particle. The different weight ratios of extract to urea were varied into five ratios: 1/5, 2/5, 3/5, 4/5 and 5/5. MCs without extract showed white color whereas MCs containing guava leaf extract was brown and the darker of color powder was attributed to the extract content in MCs. In addition the produced MCs containing soap nut extract were various shades of grey depending on the amount of the respective extract. It can conclude that increasing the extract amount corresponded to an increasing color intensity of the MCs. For %EY and %EE of MCs containing guava leaf extract, the highest %EY was the ratio of extract to urea at 4/5 and the highest %EE was at 3/5. For the prepared microcapsules containing soap nut extract the percentage encapsulation yield (%EY) and encapsulation efficiency (%EE), the highest %EY was the ratio of extract to urea at 4/5 and the highest %EE was at 3/5.

The composition of MCs containing the extract was investigated by FTIR. The FTIR spectra of the MCs containing guava leaf extract showed peaks of C=O stretching vibration at 1650 cm^{-1}, N=H stretching vibration at 1554 cm^{-1}, and C-N stretching vibration at 1245 cm^{-1} which corresponding to the peak of PUF wall. And the presence of peaks at 1444 cm^{-1} and 1118 cm^{-1} in the spectrum band of PUF MCs containing guava leaf extract correspond to the C-H bending vibration and C-C bending vibration in the spectrum of guava leaf extract. Along with

the O-H peak at 3700-3000 cm^{-1}, it showed a broader band which corresponded to the –OH group in phenolic compounds. For FTIR spectrum of the soap nut extract, MCs containing soap nut extract presented the absorption band at 1650 cm^{-1} and 1554 cm^{-1} which corresponded to C=O stretching vibration and N=H stretching vibration. Along with the O-H peak at 3700-3000 cm^{-1}, it showed a broader band which corresponded to the –OH group in saponin compounds. It showed that the guava leaf extract and the soap nut extract were successfully encapsulated in the PUF shell.

The surface morphologies of the microcapsules containing guava leaf extract and soap nut extract were observed from OM and SEM. The shape of microcapsules was found different depending on the ratio of core to wall. MCs without guava leaf extracts showed spherical particles, when the guava leaf extract was loaded at the ratio of 1/5, MCs exhibit a smaller shape. When the ratio of core to wall was increased to 3/5, the wall materials were not completely converted as the shells and the shape of MCs are irregular. For the result of the morphology of MCs containing soap nut extract, MCs at the ratio of 1/5 exhibited a larger size of particle than MCs without extract which showed spherical particles. When the ratio of core to wall, the small particles of MCs was increased and the adhesion of MCs was form to the large shape.

Then, MCs were determined the particle size distribution. The mostly particle diameter of MCs without extract was in the range of 50-140 micrometers. MCs containing guava leaf extract at 1/5 weight ratio has the diameter of MCs was in a wide ranged from 10-150 micrometers. Otherwise the diameter of the mostly MCs containing guava leaf extract at 3/5 and 5/5 weight ratio was approximately around 30-250 micrometers. The size of MCs was increased according to the weight of the extract in MCs, thus this may be caused by encapsulation of the extract by PUF. The results of the particle size distribution of the major MCs containing soap nut extract at 1/5 weight ratio was ranged from 50-140 micrometers. After the weight of soap nut extract was increased to 3/5 and 5/5 weight ratio, the particle size of MCs was in a wide range of 10-170 micrometers.

The results of TGA diagrams showed MCs containing guava leaf extract (at 1/5, 3/5 and 5/5 weight ratio of extract to urea) found weight loss temperature occurred approximate at 265 °C, 260 °C and 235 °C, respectively, because guava leaf extract was decomposed. For the

TGA results of MCs containing soap nut extract (at 1/5, 3/5 and 5/5weight ratio of extract to urea), they exhibited similar the weight loss in the temperature at 260 °C, 250 °C and 275 °C, respectively.

Cotton fabrics were finished with antibacterial agents, guava leaf extract, MCs containing guava leaf extract, soap nut extract and MCs containing soap nut extract, by screen printing method. The final color of finished cotton fabrics become the same color as the extract or MCs after treatment with extract or the MCs. The cotton fabric finished with guava leaf extract had a more intense homogeneous brown color and a smoother surface than the cotton fabric finished with MCs containing guava leaf extract. While the cotton fabric finished with MCs containing guava leaf extract showed homogenous brown spot. As well as the cotton fabrics finished with soap nut extract and MCs containing soap nut extract were homogenous light yellow and homogenous light brown spot. After the fabric samples were characterized by SEM, it was concluded that the spots were MCs which had attached on the surface.

Next, the finished fabrics were tested their antibacterial activity against *E.coli* and *S.aureus* using disc diffusion method (SN 19025-1998). Fabric finished with guava leaf extract and fabric finished with MCs containing guava leaf extract could not inhibit the growth of *E.coli*. However, the inhibition zone could be seen in fabrics finished with guava leaf extract and fabric finished with MCs containing guava leaf extract against *S.aureus*, it concluded that they could inhibit the growth of *S.aureus*. Furthermore, the fabrics finished with soap nut extract and MCs containing soap nut extract did not also show the inhibition zone against *E.coli*, but it was affected to *S.aureus*.

After, AATCC147-2004 method with modification was used to confirm the efficiency of antibacterial activity; the finished fabrics were assessed for antibacterial activity against *E. coli* and *S. aureus*. The results showed the inhibition zone around the finished fabrics against *S. aureus* but there was no zone of inhibition in any of the fabrics against *E. coli*. However, there was no growth of *E. coli* underneath the fabrics finished with guava leaf extract/ soap nut extract and the fabric finished with MCs containing guava leaf extract/ MCs containing soap nut extract. The results can concluded that all of the fabrics finished with the antibacterial agents, guava leaf extract, MCs containing guava leaf extract, soap nut extract and MCs

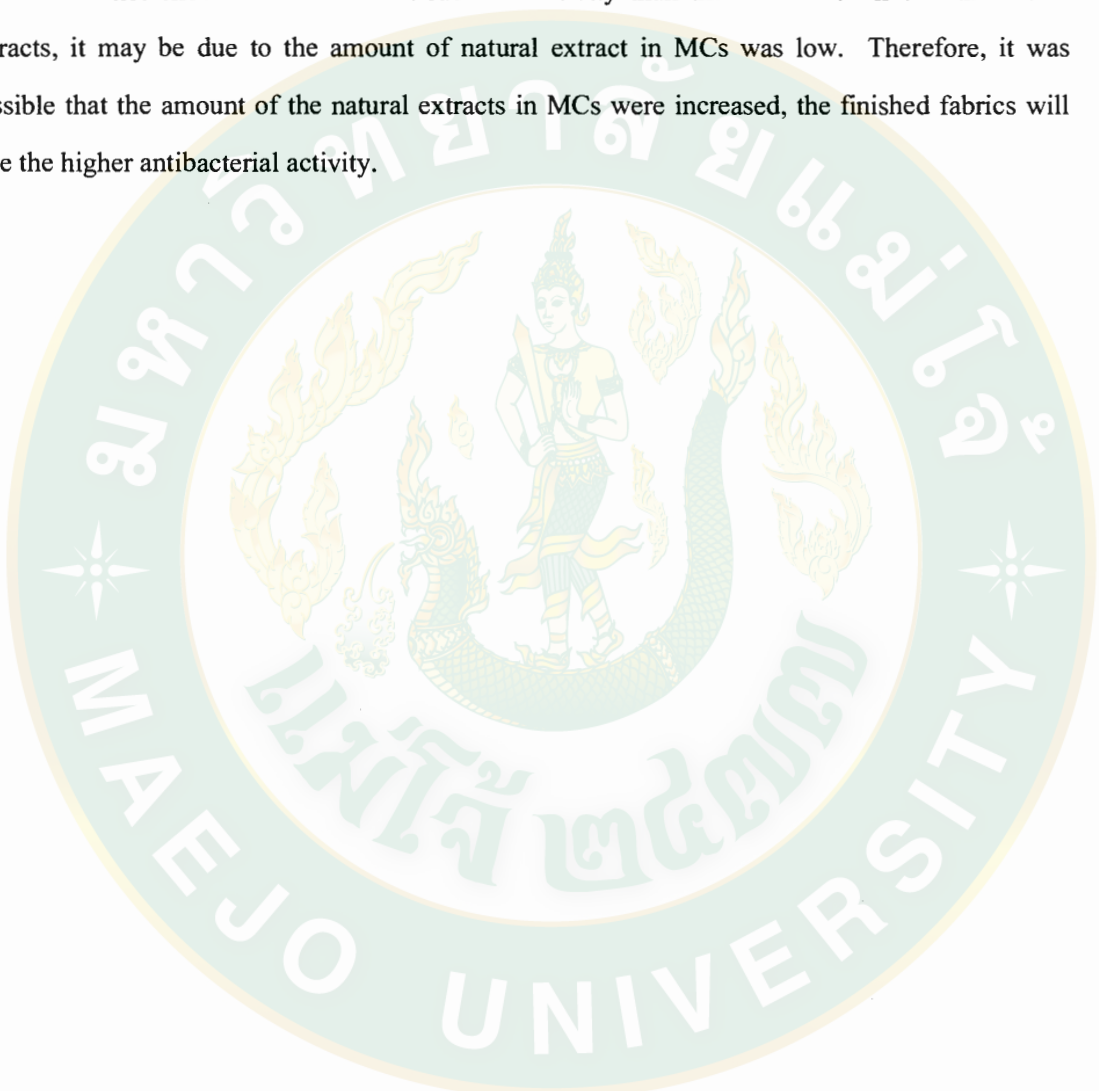
containing soap nut extract was considered efficient for the antibacterial activity against *S.aureus*, but could not inhibit the growth of *E.coli* in the present work.

The fabrics finished with the antibacterial agents were further tested for wash durability according ISO 105-C01. Following with the antibacterial activity was demonstrated as inhibition zone around the fabrics according to AATCC 147-2004 standard with modification. The fabrics finished with guava leaf extract, soap nut extract and MCs containing both extracts, showed poor wash durability against *S.aureus*. Due to there was no the inhibition zone could be obtained around the finished fabrics after washing for 1 and 5 cycles. It was indicated that the both of the direct treatment and microencapsulation method treatment on the cotton surface was easily removed by washing.

Last, both the fabric finished with extract and the fabric finished with MCs were analyzed for formaldehyde residue according to the BS EN ISO 14184-1:1999 standard. The results showed that the free formaldehyde amount on the fabric finished with extract and MCs was 0.30 and 1.51 ppm/g of fabric. Whereas, the fabrics finished with soap nut extract and MCs containing soap nut extract containing formaldehyde was approximately 0.32 and 0.69 ppm/g of fabric. The amount of formaldehyde was low so it did not affect to inhibit the growth of organism.

In this study, it was successful for encapsulation of the extracts from guava leaf and soap nut pericarps into urea-formaldehyde wall. The extracts and MCs containing the extracts were representing as the antibacterial agents against *S.aureus* in finishing textile. The cotton fabrics finished with guava leaf extract, MCs containing guava leaf extract, soap nut extract and MCs containing soap nut extract were good antibacterial activity against *S.aureus* but they were not effective with *E.coli*. It was due to the difference of cell wall structure between gram positive (*E.coli*) and gram negative (*S.aureus*) bacteria. Biswas et al. (Biswas et al., 2013) explained that gram negative bacteria were usually more resistant to the plant-origin antimicrobials and even showed no effect, compared to gram positive bacteria. Gram positive bacteria had a mesh like peptidoglycan layer which was more accessible to permeation by the extracts. Therefore, the natural extracts could destroy the cell wall of gram positive. However, the finished fabrics were poor in antibacterial activity against *S.aureus* after washing 1 and 5 cycle times. Thus, this work needs to develop the wash durability of the finished in further study,

it is suggested that the purification of natural extract to increase the specific of the active compounds, increasing the content of MCs in the recipe for finished cotton fabric, using spraying method for preparation of finished cotton fabric and encapsulation of the natural extracts by nanoencapsulation technique. Although, the fabrics finished with MCs containing natural extracts did not showed the better antibacterial activity than the fabrics finished with natural extracts, it may be due to the amount of natural extract in MCs was low. Therefore, it was possible that the amount of the natural extracts in MCs were increased, the finished fabrics will give the higher antibacterial activity.



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APPENDIX

APPENDIX A

The standard curve of gallic acid and tannic acid for calculation of phenolic content

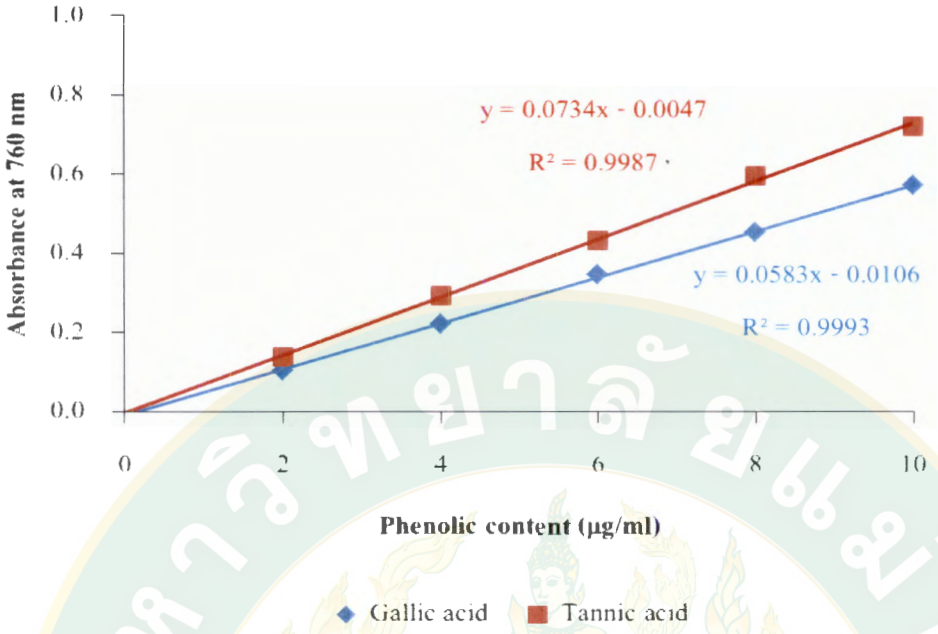

Figure A The standard curve of gallic acid and tannic acid for calculation of phenolic content

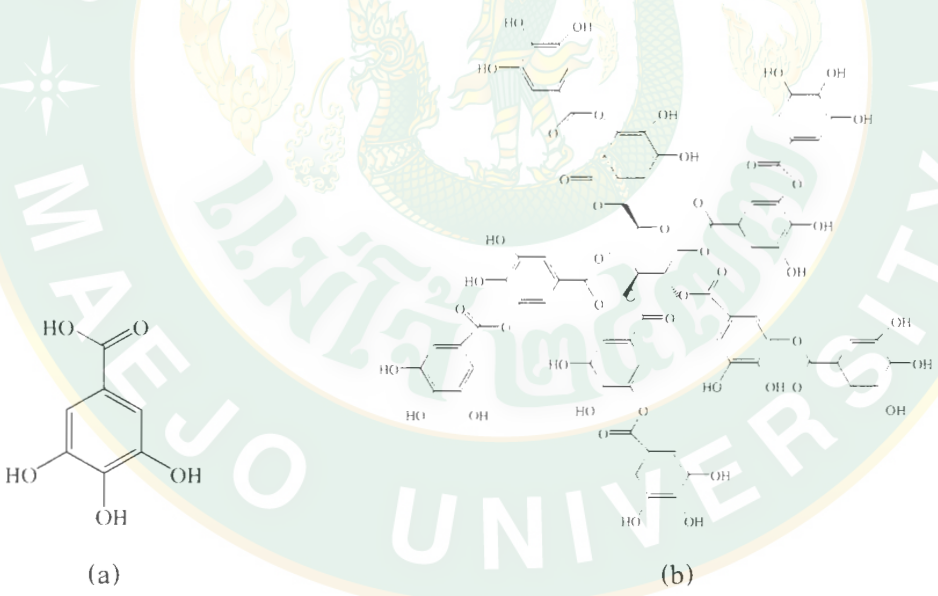

Figure B The chemical structures of (a) gallic acid and (b) tannic acid

Calculation of total phenolic content

Total phenolic content (GAE) = $(A_{760} + 0.0047) / 0.0734$

Total phenolic content (TAE) = $(A_{760} - 0.0106) / 0.0583$

APPENDIX B

The standard curve of sasarsapogenin for calculation of saponin content

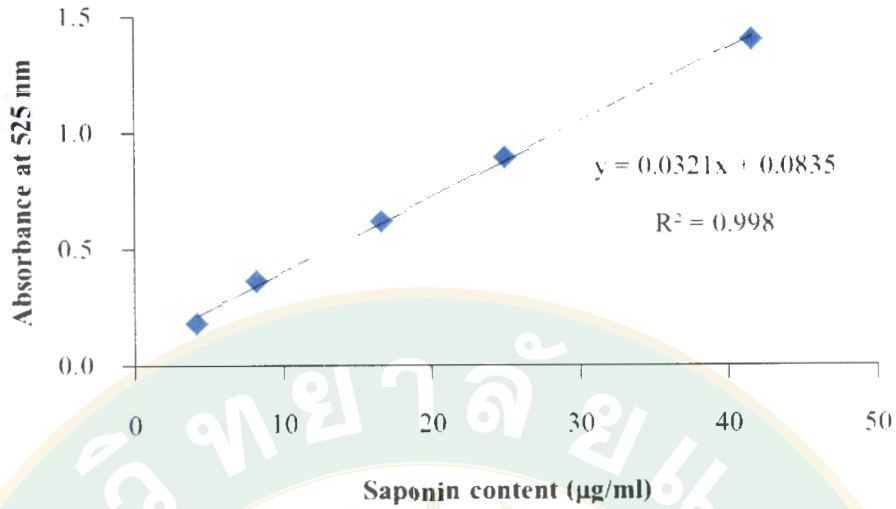

Figure C The standard curve of sasarsapogenin for calculation of saponin content

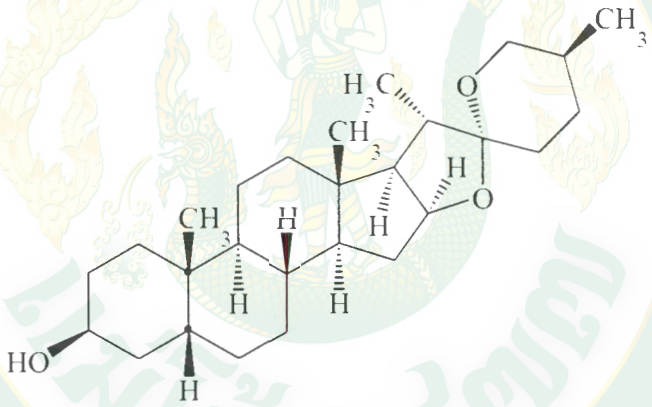

Figure D The chemical structure of sasarsapogenin

Calculation

Total saponin content = $(A_{525} - 0.0835) / 0.0321$

APPENDIX C

Encapsulation yield of MCs

Table A Yield of MCs containing guava leaf extract

Wt ratio of	Weight of MCs (g)			Weight of total
extract/urea (g/g)	1	2	3	materials (g)
0/5	4.39	4.26	4.52	10.68
1/5	4.27	4.03	3.72	11.68
2/5	4.40	5.12	4.50	12.68
3/5	4.85	5.52	5.19	13.68
4/5	5.86	6.07	6.05	14.68
5/5	5.51	5.70	5.37	15.68

Table B Encapsulation yield of MCs containing guava leaf extract

Wt ratio of	%EY			
extract/urea (g/g)	1	2	3	Avg
0/5	41.10	39.89	42.32	41.10 ± 1.23
1/5	36.56	34.50	31.84	34.30 ± 2.36
2/5	34.70	40.38	35.49	36.86 ± 3.04
3/5	35.45	40.35	37.94	37.91 ± 2.46
4/5	39.92	41.35	41.21	40.83 ± 0.77
5/5	35.14	36.35	34.25	35.25 ± 1.05

Calculation

Example at 1/5 ratio

$$\text{Encapsulation yield (\%)} = \frac{\text{Wt of obtained MCs} \times 100}{\text{Wt of total materials}}$$

$$= \frac{4.39 \times 100}{10.68}$$

$$= 41.10$$

Table C Yield of MCs containing soap nut extract

Wt ratio of	Weight of MCs (g)			Weight of total
extract/urea (g/g)	1	2	3	materials (g)
1/5	2.83	2.83	2.88	11.68
2/5	3.16	3.10	3.67	12.68
3/5	5.54	5.30	4.96	13.68
4/5	9.52	9.77	9.73	14.68
5/5	9.15	9.42	9.65	15.68

Table D Encapsulation yield of MCs containing soap nut extract

Wt ratio of	%EY			
extract/urea (g/g)	1	2	3	Avg
1/5	24.23	24.23	24.66	24.37 ± 0.24
2/5	24.92	24.45	28.94	26.10 ± 2.48
3/5	40.49	38.74	36.26	38.50 ± 2.11
4/5	64.85	66.55	66.28	65.89 ± 0.91
5/5	58.35	60.08	61.54	59.99 ± 1.59

APPENDIX D

Encapsulation efficiency of MCs

Table E Encapsulation efficiency of MCs containing guava leaf extract

Wt ratio of	%EE			
extract/urea (g/g)	1	2	3	Avg
1/5	78.58	88.44	83.23	83.42 ± 4.93
2/5	76.42	85.43	84.53	82.13 ± 4.96
3/5	85.07	84.69	83.22	84.33 ± 0.98
4/5	86.66	85.71	79.36	83.91 ± 3.97
5/5	82.62	78.07	77.70	79.46 ± 2.74

Table F Encapsulation efficiency of MCs containing soap nut extract

Wt ratio of	%EE			
extract/urea (g/g)	1	2	3	Avg
1/5	28.19	28.78	24.85	27.27 ± 2.12
2/5	50.06	59.32	49.28	52.89 ± 5.59
3/5	55.78	54.60	56.89	55.76 ± 1.14
4/5	59.42	60.11	60.25	59.93 ± 0.45
5/5	63.40	62.33	64.56	63.43 ± 1.12

Calculation

Encapsulation efficiency (%) = $[(w_1 - w_2) / w_1] \times 100$

w_1 = wt of extract used for preparation microcapsules

w_2 = wt of extract left in supernatant after preparation of microcapsules

w_2 = wt of saponin x dilution x Volumn of supernatant / 246

= $[(A_{525} - 0.0835) / 0.0321]$ x dilution x Volumn of supernatant /246

Example at1/5 ratio of MCs containing soap nut extract

$W_2 = [(0.337 - 0.0835) / 0.0321] \times 100 \times 225/ 246 = 722.3$ mg

EE (%) = $[(1.0058 - 0.7223) / 1.0058] \times 100 = 28.19$

APPENDIX E

Mean diameter of MCs

Table G Mean diameter of MCs

$Wt_{extract}/W_{urea}$	Mean diameter (μm)			
	1	2	3	Avg
MCs without extract				
0/5	36.54	36.65	36.65	36.65 ± 0.06
MCs containing guava leaf extract				
1/5	48.76	48.81	48.87	48.81 ± 0.06
3/5	86.98	86.72	87.24	86.98 ± 0.26
5/5	80.73	81.10	80.46	80.76 ± 0.32
MCs containing soap nut extract				
1/5	15.56	15.27	15.50	15.44 ± 0.15
3/5	35.60	35.29	35.35	35.41 ± 0.16
5/5	47.41	47.49	47.80	47.57 ± 0.21

APPENDIX F

The calibration curve for determination of formaldehyde amount

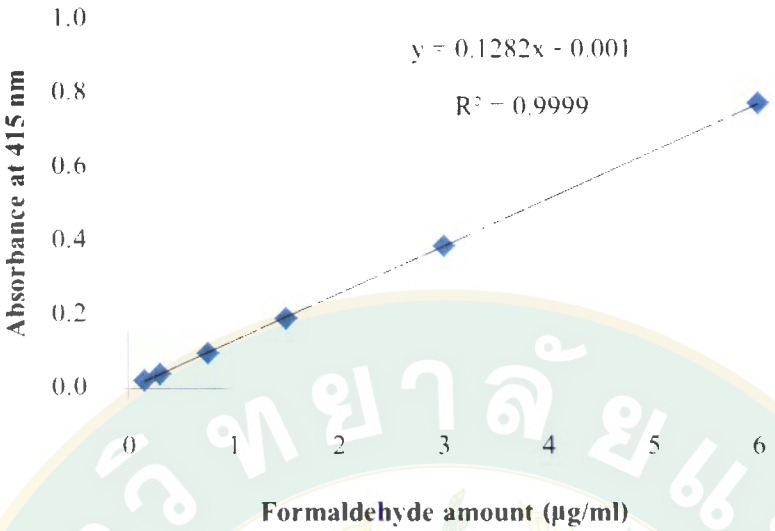

Figure E The calibration curve for determination of formaldehyde amount

Table H Formaldehyde amount on finished fabrics

Finished fabrics	Formaldehyde amount (ppm)			
	1	2	3	avg
Guava	0.30	0.31	0.28	0.30 ± 0.016
MCs-Guava	1.47	1.48	1.59	1.51 ± 0.068
Soap nut	0.33	0.33	0.31	0.32 ± 0.009
MCs-Soap nut	0.70	0.67	0.70	0.69 ± 0.018

Calculation

Formaldehyde amount = $[(A_{415} - 0.001) / 0.1282] /$ wt of fabric for testing

= $[(0.018 - 0.001) / 0.1282] / 0.5$

= 0.30 µg/ml/g of fabric

= 0.30 ppm/g of fabric

APPENDIX G

VITA

VITA

NAME	Miss Jiraporn Ketwaraporn	
DATE OF BIRTH	January 27, 1982	
EDUCATION	2000-2004	B.Sc. (Chemistry), Department of Chemistry, Faculty of Science, Naresuan University, Phitsanuloke, Thailand.
	2007-2010	M.S. (Biotechnology), Division of Biotechnology, the Graduate School, Chiang Mai University, Chiang Mai, Thailand.
WORK EXPERIENCE	2004-2007	Chemist, Winson Ink Co., Ltd., Bangkok, Thailand.
	2010-2012	Lecturer, Uttaradit Rajabhat University, Uttaradit, Thailand

PROCEEDINGS

1. Ketvaraporn, J. and Kongdee, A. 2013. Microencapsulation of *Psidium Guajava* Linn. leaf extract. In **2013 International Textiles and Costume Congress**. Kasetsart University. pp. 138-143.
2. Ketvaraporn, J. and Kongdee, A. 2014. Preparation of microcapsules containing *Sapindus rarak* DC. extract. In **MACRO 2014**. Chiang Mai: IUPAC.

PUBLICATION

1. Katewaraphorn, J. and Aldred, K. A. 2016. A study of Microcapsules containing *Psidium Guajava* leaf extract for antibacterial agent on cotton fabric. **International Journal of Chemistry Engineering and Applications** 7(1):27-31.

MICROENCAPSULATION OF PSIDIUM GUAJAVA LINN. LEAF EXTRACT

Jiraporn Ketvarporn[1], Arunee Kongdee[1]*

[1]*Program in Chemistry, Maejo University, Chiang Mai, 50290, Thailand*

Abstract: *Psidium guajava* Linn. leaf extracts containing phenolic compounds were known for antimicrobial activity. The encapsulation of *Psidium guajava* Linn. leaf extracts has potential for application in antimicrobial finishing textile. The aim of this study was to encapsulate leaf extracts *Psidium guajava* Linn. by in situ interfacial polymerization of urea-formaldehyde. The different concentrations of encapsulants were applied to optimize the highest loading capability. The quality of microcapsules was evaluated by determining total amount of phenolic compounds and residue of radical formaldehyde. Microcapsules obtained were further characterized using FT-IR and SEM. The present study demonstrates potential to produce antimicrobial microcapsules for textile application.

Introduction

Psidium guajava, commonly known as guava, belongs to family of *Mystraecea* and is native plant throughout in the South America, European, Africa and Asia. Guava leaves are rich in flavonoids and phenols including other compounds such as terpenoids, tannins, essential oils, carotenoids, chlorophyll and saponins [1,2]. The extract of guava leaves was found to inhibit the growth of *Bacillus cereus*, *Escherichia coli*, *Proteus* spp., *Pseudomonas aeruginsa*, *Salmonella enteritidis*, *Shigella* spp. and *Streptococcus mutans* in vitro agar test [2]. Its antimicrobial activities were beneficial application in antimicrobial finishing textile. The consumers are now increasingly aware of the hygienic life style so the antimicrobial property of fabric is considered to be an important. Especially, commercial products are currently trend to show natural or herbal products because of their environmental friendly [3]. By the way the interesting in textiles with new properties was high added value, such as cosmetic textiles, functional textiles and medical textiles.

Nowadays, the scientific advance is used for the development of innovative textile products including insect repellants, dyes, vitamins, phase-change materials, fragrances and skin softeners, antimicrobial agents and medical applications [4]. A number of commercial applications of microencapsulation in textile are growing. Microencapsulation technique is widely spread technique using for developing new product. It is a process by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of a polymer [5]. This technique is used to protect active agents from the environment and to control release of the active components for long-acting release [6]. The aim of this work was the encapsulation of guava leaf extracts into urea-formaldehyde microcapsules by in situ polymerization to be developing an ecofriendly natural antimicrobial finish in textiles.

Keywords: urea-formaldehyde, *Psidium guajava*, microcapsule, polymerization, antimicrobial activity, textile

*Corresponding author: arunee.k@mju.ac.th

Materials and methods

2.1 Materials

Urea, 36% formaldehyde and acetic acid were obtained from VMR International S.D.S. Ammonium acetate and Folin-Ciocalteu reagent were purchased from QRëC®. Polyvinyl alcohol was from Ajax Finechem Pty Ltd. Ammonium chloride was from RANKEM. Resorcinol was from HIMEDIA Laboratories Pvt. Ltd.

2.2 Preparation of extracts

The fresh leaves of *Psidium guajava* L. were collected from Chiang Mai. The leaves were washed to remove debris and dried at 50°C for 24 h in an oven. After completely dried, the leaves were crushed into small pieces. Samples ware extracted with distilled water in a ratio of 1:15 at 60°C for 1 h. The extracts were sieved through cheesecloth and followed with Whatman No.3 filter paper. The filtrates were concentrated by evaporating water and freeze-dried at -20°C. Crude extracts were stored in 4°C for further application.

2.3 Determination of total phenolic content

Total phenolic compounds in the extracts from guava leaves were determined using Folin-Ciocalteu reagent assay which was modified from Lee [7]. Briefly, crude extracts were dissolved in distilled water at a final concentration of 0.1 mg/ml prior to test. An aliquot (0.5 ml) of samples was mixed with 0.5 ml of Folin-Ciocalteu reagent and 4.0 ml of 20%(w/v) sodium bicarbonate solution, respectively. After 30 min maintenance at room temperature, the absorbance was measured spectrophotometrically at 760 nm. The experiment was run in triplicate. Tannic acid (0-10 μg/ml) was used as the standard for the calibration curve. The total phenolic content was expressed in terms of tannic acid equivalent (mg/g) of the extracts.

2.4 Preparation of microcapsules containing *Psidium guajava* extracts

Poly(urea-formaldehyde) microcapsules containing guava leaf extracts were prepared by in situ polymerization according to the procedure of Suryanarayana [8] with modifications. Firstly, 5 g of urea, 0.5 g of ammonium chloride, 0.5 g of resorcinol and 260 ml of deionised water were mixed in 500 ml beaker. Then, the mixture was added 10 ml of 5%wt aqueous solution of polyvinyl alcohol (PVA). After the pH was adjusted to 3.5 with 5%wt solution of hydrochloric acid, the extracts were loaded in the mixture and left for 10 min for stabilization. Then, 12 ml of 36%wt formaldehyde solution was added. The reaction was constantly heated at 55°C and carried out under stirring at 800 rpm for 4 h. The prepared microcapsules were filtered under vacuum and thoroughly washed with deionised water. Microcapsules were dried at room temperature and stored under vacuum.

2.5 Determination of total phenolic content in microcapsules

For the determination of total phenolic compounds in microcapsules, 0.1 g of microcapsules was crushed using pestle and mortar and rinsed with 10 ml of deionised water. The dispersions were ultra-sonicated at room temperature for 1 h and centrifuged at 3500 rpm for 15 min. The supernatant was collected and quantified for phenolic compounds content according to **2.3**. Total phenolic content were reported as the amount of phenolic compounds (μg) per gram of microcapsules.

2.6 Determination of free formaldehyde

Method for the determination of free formaldehyde is 2,4-pentanedion method according to BS EN ISO 14184-1:1999 part I Annex A [9]. Briefly, 0.5 g of microcapsule samples was firstly put into 250 ml flask and followed with 20 ml of deionised water. The flask was covered with stopper and heated in a water bath at 40°C for 1 h with shaker. Then, the warm solution was filtered into another flask through a Whatman filter. After 5 ml of solution sample was transferred into a tube, 5 ml of acetylacetone reagent solution was added into the tube and covered with stopper and shake it. The mixture was kept first in a water bath at 40°C for 30 min and then was left at room temperature for 30 min. Blank reagent was prepared by adding 5 ml of acetyl acetone reagent solution to 5 ml of deionised water and treated it the same way. The absorbance was measured at 415 nm using a spectrophotometer (U2001 Hitashi, Japan). Formaldehyde solutions (0.15-3.00 µg/ml) were prepared as the calibration solutions. Each test was performed in triplicates.

2.7 Analysis of microcapsules

The morphology and surface of microcapsules was observed by scanning electron microscope (SEM, 5410LV JEOL). One drop of the microcapsule dispersion was placed on a stainless steel stub and dried at 50°C for 1 h. The sample was sputtered with a thin layer of gold. The composition of microcapsules and extract were obtained to identify the chemical structure using a FTIR spectrophotometer (Perkin Elmer), was prepared by grinding the sample with a potassium bromide (KBr) and analysed in KBr pellet form.

3. Results

3.1 Total phenolic content

The crude extracts of guava leaves were obtained from hot water extraction and determined the quantitative of phenolic compounds by Folin-Ciocalteu assay which is a widespread assay. The blue pigments, molybdotungstophosphate blue, were the results of the oxidation of phenols in alkaline solution by the yellow molybdotungtophosphoric heteropolyanion reagent. The depth of blue color depended on the qualitative composition of phenolic content [4]. Total phenolic content in guava leaf extracts were 38.8 mg TAE/g extract.

3.2 Microcapsule preparation

The process of urea-formaldehyde microcapsule preparation has been described by Suryanarayana. Polymerization of urea and formaldehyde was resulted to obtain methylol ureas as the wall materials [8]. In the preparation of microcapsules, the weight ratio between core to wall (W_{core}/W_{wall}) was varied to be 1/5, 2/5, 3/5, 4/5 and 5/5, respectively in this study. Afterwards, total amount of phenolic compounds was determined by using Folin-Ciocalteu assay and the amount of residue formaldehyde was determined following the method of BS EN ISO 14184-1:1999 part I Annex A. The results as shown in Table 1 were indicated that the amount of phenolic compounds was increased with increasing the ratio of core to wall. Formaldehyde amount were found varied with increasing the ratio of core to wall.

Table 1. Total amount of phenolic compounds and formaldehyde amount

W_{core}/W_{wall}	Total amount of phenolic compounds (μg/g)	Formaldehyde amount (ppm)
0/5	0	6.1 ± 2.6
1/5	49.8 ± 22.5	14.7 ± 1.5
2/5	103.8 ± 33.1	19.6 ± 0.5
3/5	215.5 ± 55.7	24.2 ± 0.9
4/5	330.8 ± 26.9	25.5 ± 2.4
5/5	451.7 ± 22.8	30.4 ± 4.1

3.3 Microcapsule surface morphology

In the preparation of the guava leaf microcapsules, the ratio of core to wall (W_{core}/W_{wall}) is important to the shape and size of the final microcapsules. The surface morphologies of obtained microcapsules are illustrated in Figure 1. Microcapsules without guava leaf extracts show spherical particles in Figure 1a. When the guava leaf extracts is loaded at the ratio of 1/5, microcapsules exhibit a smaller shape. Besides, compared with 1c, the wall materials could not simultaneously deposit onto the drops to form complete sphere. Furthermore, when the ratio of core to wall are increased to 3/5 (Figure 1d), the wall materials are not completely converted as the shells and the shape of microcapsules are irregular. It may be due to the overloading of the core.

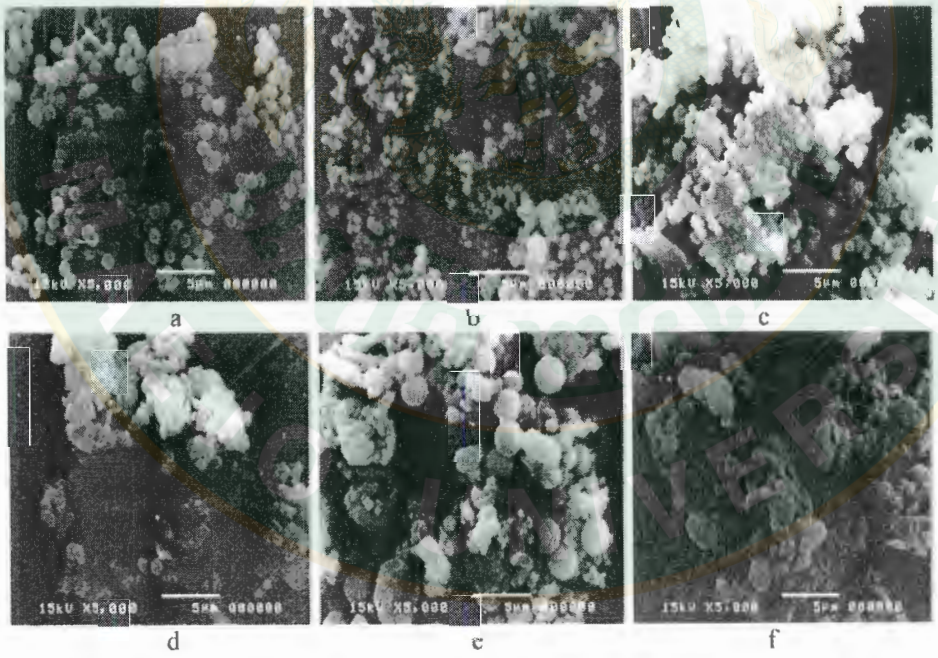

Figure 1. SEM micrograph of urea-formaldehyde microcapsules were prepared with the different ratio of core to wall, 0/5, 1/5, 2/5, 3/5, 4/5 and 5/5 respectively (a-f).

FTIR spectrum of guava leaf extract (Figure 2b) shows absorption bond at 1714 cm^{-1} which corresponding to C=O stretching in phenolic compounds. Whereas spectra of urea-formaldehyde microcapsules (Figure 2a) and urea-formaldehyde microcapsules containing guava leaf extracts (W_{core}/W_{wall} : 1/5) (Figure 2c) show similar pattern. However, higher intensity of absorption band

at 1655 cm^{-1} in spectrum of urea-formaldehyde microcapsules containing guava leaf extracts is observed C=O group in phenolic compounds may high %T, apart from C=O groups in urea-formaldehyde resin. The C=O groups are shifted from 1714 cm^{-1} to 1655 cm^{-1} due to their conjugation with NH_2 groups. Moreover, the presence of absorption band at 1548 cm^{-1} assign to N-H bending of amide.

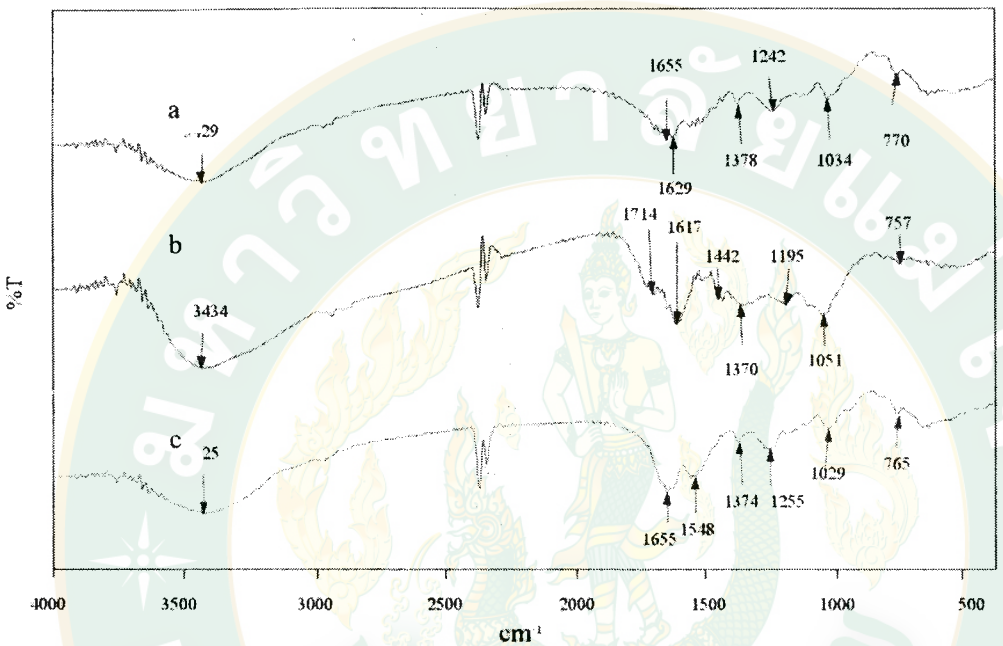

Figure 2. FTIR spectrum of a) urea-formaldehyde microcapsules without the extracts, b) guava leaf extracts and c) urea-formaldehyde microcapsules containing guava leaf extracts.

4. Conclusions

Microcapsules containing guava leaf extracts were constructed with using urea-formaldehyde resin as shell by in situ polymerization. Total amount of phenolic compounds and residue formaldehyde were increased when the content of guava leaf extracts in microcapsules increased. The surface morphologies of microcapsules were found different depending on the ratio of core to wall. Moreover, the results of FTIR were used to confirm the encapsulation of guava leaf extracts in microcapsules due to the disappeared of 1714 cm^{-1} and high %T of C=O absorption bond at 1655 cm^{-1}. These obtained microcapsules were further applied for finishing of cotton by pad-dry-cure method and fabric finished with microcapsules was assessed antimicrobial activity.

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Preparation of Microcapsules Containing *Sapindus rarak* DC. Extract

Jirapom Ketvaraporn and Arunee Kongdee

Program in Applied Chemistry, Faculty of Science, Maejo University, Chiang Mai 50290
Phone: 66 (0) 60 48 5..; E-Mail: arunee.k@mju.ac.th

Introduction

Microencapsulation technique is a process by which very tiny droplets or particles of liquid or solid materials are coated or surrounded with a continuous film of polymer [1]. Commonly, this technique was used to protect the active ingredient from the environment and to control release of the active ingredient for long acting release [2].

Sapindus rarak DC. (Sapindaceae) or soapnut is a tall tree which originates in South East Asia and now widely distributes in Asia and Africa. Its fruits have pericarps which are soft and brown color and become dark brown when it is dried. The pericarps contain a foaming agent and have been used as a natural soap for cleaning [3]. Whole of *S. rarak* DC. fruit extract contains saponins. Saponins are bioactive compounds and the chemical structure of saponins is composed of glycone (saccharide side groups) and aglycone (known as sapogenin). Commonly, saponins can be classified into two groups according to the chemical character of the aglycone. First group is steroidal saponins are mainly compounds containing 27 carbon atoms, for example, spirostan and furostan. Second group is triterpenoid saponins which contain aglycone with 30 carbon atoms or their nonderivatives. The most commonly occurring are pentacyclic oleanans and tetracyclic dammarans [4]. The chemical structure of some identified saponins from *S. rarak* DC. fruit pericarps is monodesmoside triterpenoid saponins that possess hederagenin as the aglycone. Glycone part is arabinose attached to hederagenin and rhamnose and xylose as sugar residues are attached to arabinose [5].

Saponins have several biological and pharmacological properties such as anti-inflammatory, antifungal antiyeast, antibacterial antimicrobial and antiparasasitic activity [6]. These activities were interested for applications in textile to produce a functional fabric.

The aim of this work is the encapsulation of *S. rarak* DC. extract into urea-formaldehyde microcapsules by *in situ* polymerization to be developing an ecofriendly natural antimicrobial finish in textiles.

Experimental

Materials. *Sapindus rarak* DC. dried fruits used as core material were purchased from local market in Chiang Mai, Thailand. Urea and 36 wt% formaldehyde used as wall materials were obtained from VMR International S.D.S., Belgium. Polyvinyl alcohol used as surfactant was purchased from Ajax Finechem Pty Ltd., Australia. Ammonium chloride was purchased from RFCL, India. Resorcinol was purchased from Himedia Laboratories Pvt. Ltd., India. Sarsasapogenin was purchased from Sigma, Mexico. Vanillin was purchased from Sigma Aldrich, French. Sulphuric acid (98% purity) purchased from Merck, Germany.

Preparation of extract. The dried pericarps of *S. rarak* DC. were blended into small pieces. Samples were extracted with distilled water at a ratio of 1:15 at 60 °C for 1 hr. Next, the extracts were filtered through cheese cloth 2 times. The filtrates were concentrated by evaporating water and freeze-dried at -20 °C. Last, crude extracts were stored in 4 °C for further application.

Determination of total saponins content. The procedure was performed according to Shiau *et al.* [7]. 0.5 ml of sample solution and 0.5 ml of 8 wt% vanillin solution in ethanol (fresh solution) were mixed together. After that, 5.0 ml of 72 wt% sulfuric acid was added and thoroughly mixed in an ice water bath. The mixture was heated in water bath at 60 °C for 10 min and then was cooled in ice water bath. Finally, the solution was spectrometrically

measured at 535 nm by UV-Visible spectrophotometer (U-2001, Hitachi, Japan).

Preparation of microcapsules. Poly(urea-formaldehyde) microcapsules containing *S. rarak* DC extract were prepared by *in situ* polymerization according to the procedure of Suryanarayana C *et al.* [8] with modifications. Firstly, 5 g of urea, 0.5 g of ammonium chloride, 0.5 g of resorcinol and 260 ml of distilled water were mixed in 500 ml beaker. Then, the mixture was added by 10 ml of 5%wt aqueous solution of polyvinyl alcohol (PVA). After the solution pH was adjusted to 3.5 by 5 wt% hydrochloric acid solution. *S. rarak* DC. extract (weight ratio of extract to urea: 0/5, 1/5, 2/5, 3/5, 4/5 and 5/5) was loaded in the mixture and left for 10 min for stabilization. Then, 12 ml of 36 wt% formaldehyde solution was added. The reaction was constantly heated at 55°C and carried out under stirring at 800 rpm for 4 hr. The prepared microcapsules were filtered and the supernatant was collected to determine %EE (section 2.5). Microcapsules were thoroughly washed with distilled water and dried at room temperature and stored under vacuum.

Determination of encapsulation yield (EY) and encapsulation efficiency (EE). Encapsulation yield was calculated as the percentage of weight of obtained microcapsules and weight of total materials.

$$\%EY = \frac{\text{wt of obtained microcapsules}}{\text{wt of total materials}} \times 100$$

when wt of total materials is total wt of urea, resorcinol, PVA, formaldehyde and extract.

Encapsulation efficiency was calculated as the percentage of weight of the extract was entrapped in microcapsules and weight of the extract was used.

$$\%EE = \frac{w_1 - w_2}{w_1} \times 100$$

w_1 = wt of extract used for preparation microcapsules
w_2 = wt of extract left in supernatant after preparation of microcapsules

Scanning electron microscopy (SEM). The morphology of microcapsules was observed by scanning electron microscope (5410LV JEOL). One drop of the microcapsule dispersion was placed on a stainless steel stub and dried at 50°C for 1 h. The sample was sputtered with a thin layer of gold.

Results and Discussion

S. rarak DC. extract was obtained from hot water extraction and determined total saponins by vallin-sulfuric acid assay were 246 mg/g extract. Encapsulation of *S. rarak* DC. extract in a poly(urea-formaldehyde) wall was carried out under acidic condition. Urea and formaldehyde are soluble in water. Since the pH is changed to acidic and heated to 55°C, urea and formaldehyde reacted and resulting in poly(urea-formaldehyde). The initial step of polymerization, urea-formaldehyde molecule is rich with polar groups and is water compatible. Product in this step is called methyol ureas. After that, the number of polar groups will gradually reduce when the molecular weight of polymer increases. Finally, the hydrophylicity of poly(urea-formaldehyde) molecule is reduced leading to separation from aqueous phase and obtained droplets [8].

Microcapsules without *S. rarak* DC extract and microcapsules containing *S. rarak* DC extract were produced as showed in figure 1. Microcapsules without *S. rarak* DC extract shown white color, whereas microcapsules containing *S. rarak* DC extract were grey. Color strength is dependent on the amount of extract.

Table 1. Encapsulation yield and encapsulation efficiency of microcapsules

wt ratio of extract to urea	%EY	%EE
1/5	24.42 ± 0.29	27.27 ± 2.12
2/5	24.67 ± 0.32	52.89 ± 5.59
3/5	38.37 ± 2.97	55.76 ± 1.14
4/5	65.54 ± 0.99	59.92 ± 0.45
5/5	59.22 ± 2.82	63.43 ± 1.12

The prepared microcapsules were further analyzed. The percentage encapsulation yield (%EY) and encapsulation efficiency (%EE) were determined and the results were shown in table 1. %EE and %EY increased when increasing the amount of extract. The highest %EE found at 5/5, whereas the highest %EY found at 4/5. Increasing the amount of extract was 5/5, %EY decreased. The highest %EY should be at 5/5. This result may be resulted from tiny microcapsules probably passed the filter paper due to the size of microcapsules containing *S. rarak* DC. at 5/5 was smallest (as seen in figure 2.). Thus, the weight of microcapsules was less than the actual weight.

The morphology microcapsules without extract showed spherical particles in figure 2a. When *S rarak* DC extract was loaded at the ratio of 1/5, microcapsules exhibit a larger size (figure 2b). Furthermore, when the ratio of core to wall were increased (figure 2c-2f), microcapsules are smaller. This may be affected by surfactant property of saponins. The size of microcapsules decreased with an increasing the amount of extract which was corresponding to the work of Maryanti *et al.* [9]. They had synthesized of ZnO nanoparticles by hydrothermal method in aqeous rinds extracts of *S. rarak* DC. In addition, they revealed that the morphology of ZnO was dependent on the concentration of *S. rarak* DC. rinds extract and the ZnO morphology is influenced by the presence of the natural surfactant.

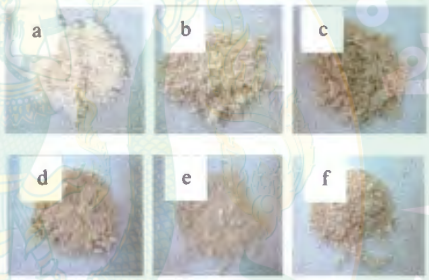

Figure 1. The images of urea-formaldehyde microcapsules synthesized with the *S. rarak* DC. extract/urea weight ratio at a) 0/5 b) 1/5 c) 2/5 d) 3/5 e) 4/5 and f) 5/5.

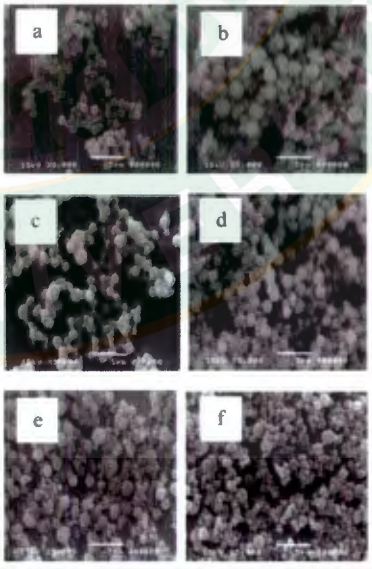

Figure 2. Scanning electron microscopy (SEM) images of urea-formaldehyde microcapsules synthesized with the *S. rarak* DC. extract/urea weight ratio at a) 0/5 b) 1/5 c) 2/5 d) 3/5 e) 4/5 and f) 5/5

Conclusions

Microcapsules containing *S. rarak* extract has been successfully prepared by using urea-formaldehyde resin as wall through *in situ* polymerization. Encapsulation yield (%) was increased with increasing the ratio of W_{core}/W_{wall} except at the ratio of 5/5. Encapsulation efficiency (%) was highest at the ratio of 5/5. The morphology of microcapsules was spherical and microcapsules were smaller when the ratio of core to wall was increased.

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Effect of rubber content on mechanical properties of poly(lactic acid) blended with masticated natural rubber

Rudee Jaratrotkamjorn and Varaporn Tanrattanakul*

Bioplastic Research Unit, Department of Materials Science and Technology, Faculty of Science
Prince of Songkla University, Songkla 90112, Thailand
Phone: 0-7428-8362, Fax: 0-7444-6929, E-Mail: varaporn.t@psu.ac.th

Introduction

Polylactide or poly(lactic acid) (PLA) is a biodegradable aliphatic polyester derived from renewable resources. PLA shows good properties such as clarity, good strength and stiffness. However, it is quite brittle, i.e., low notched impact strength (< 3 kJ/m2) and low elongation at break (< 5%) [1] which is significant limitation of PLA comparing with conventional thermoplastics. To improve the toughness of PLA, PLA has been blended with some polymers, i.e., ethylene acrylate copolymer [2], poly(lactide-*b*-butadiene-*b*-lactide) triblock copolymer [3], styrene ethylene butylene styrene triblock copolymer-g-polylactic acid [4], poly(ethylene-glycidyl methacrylate) [5], poly(ε-caprolactone) [6], thermoplastic polyurethane [7], polyurethane [8] and poly(ether)urethane [9].

Natural rubber (NR) is an eco-friendly rubber and derived from a renewable resource. NR can be used as a toughening agent due to its high molecular weight and very low glass transition temperature (-70 °C). Our previous work successfully enhanced the impact strength of PLA by blending with natural rubber (NR) when NR content was 10wt% [1] and this result agreed with other works [10,11]. There have been many researchers working on the enhancement of the compatibility of the PLA/NR blend. The modified NR was a promising material for this purpose, e.g., NR grafted with poly(methyl methacrylate) [1], NR grafted with poly(vinyl acetate) [12] and NR grafted with glycidyl methacrylate [13]. Recently, our groups synthesized diblock and triblock copolymers from PLA and NR for using as the compatibilizer and the toughening agent. Although these modified NRs could act as the toughening agent of PLA or the compatibilizer of the PLA/NR blend, their synthesis methods made them become less friendly for a user and less environmental friendly. One simple method for increasing the impact strength of the PLA/NR blend was the mastication of NR by a two-roll mill [1]. The achievement of this method was due to the molecular weight of NR decreased significantly and perhaps its low viscosity matched to the viscosity of PLA. In the previous work [1], only the blend containing 10 wt% NR was studied.

The aim of the present study was to determine the effect of the masticated NR contents on the impact strength and tensile properties of the PLA containing higher NR content, 10-20 wt% NR. The effect of mastication of the molecular weight and Mooney viscosity of NR was investigated. The NR particle diameters in the blends were demonstrated as well. The present study showed that the effect of NR content was more dominant than the rubber mastication. The results substantiated the previous works that 10 wt% NR was an optimum content for PLA.

Experimental

Materials

PLA Ingeo™ 2002D was produced by Natureworks LLC. It contains 96.0% of L-lactide configuration and 4.0% of D-lactide configuration. NR, STR5 CV60 was produced by Jana Concentrated Latex Co., Songkla, Thailand.

Polymer Blend Preparation

NR was masticated with a two-roll mill (6 inch - diameter) at room temperature which controlled by the water cooling inside both rollers. The masticated NR was cut into small pieces and dry mixed with PLA pellets in a container. Mechanical blending between PLA and NR was performed in a twin screw extruder, Prism® TSE16TC at 160 °C. The extrusion was done twice to obtain

A Study of Microcapsules Containing *Psidium Guajava* Leaf Extract for Antibacterial Agent on Cotton Fabric

Jiraphorn Katewaraphorn and Arunee Kongdee Aldred

Abstract*—*Psidium Guajava* Linn. leaf extract containing phenolic compounds are known for antimicrobial activity. This study's objective was to prepare antibacterial cotton fabric by using microcapsules containing *Psidium guajava* Linn. leaf extract. Microcapsules containing *Psidium guajava* Linn. leaf extract were prepared by *in situ* polymerization using urea and formaldehyde for encapsulation. Both *Psidium guajava* Linn. leaf extract and microcapsules containing *Psidium guajava* Linn. leaf extract have been applied to cotton fabric by direct printing with a binder. The qualitative antibacterial assessment of the fabric was performed according to AATCC 147-2004 against *Escherichia coli* and *Staphlococcus aureus* as test organisms. The antibacterial tests proved that the cotton fabric finished with microcapsules containing *Psidium guajava* Linn. leaf extract showed antibacterial activity against *Staphlococcus aureus*, but was not effective against *Escherichia coli

***Index Terms*—Poly(urea-formaldehyde), microcapsule, *Psidium guajava* Linn., antibacterial textile.**

I. Introduction

Currently, scientific advancement is used for the development of innovative textile products to produce functional textiles inclusive of fragrances, dyes, insect repellants, phase-change materials, antimicrobial agents, and fire retardant. The consumer is increasing in awareness and concern for the safety of products [1]. Thus, there is a need to develop textiles that are resistant to microbes as the textile substrates find various applications such as masks, hospital covers, and surgical gowns apart from conventional apparel usage [2]. Various antimicrobial technologies have been developed to protect various materials from microbial damage and to prevent microbial infection [3]. Hence, textiles are treated with various compounds. Many commercial products are currently available in the market with a range of antimicrobial properties, under different trade names of the textile industry. Most of the products are made from synthetic agents; for example, organo-metallics, phenols, quaternary ammonium salts and organosilicons. Even though the synthetic antimicrobial agents show high effectiveness for inhibiting the microbes, they are hazardous to human health [4]. Different chemicals and heavy metals are non-biodegradable. For this reason, natural extracts for finished fabrics are being considered as an alternative antibacterial agent for this work.

Psidium guajava Linn. (family Myrtacae) is commonly called guava [5]. Guava has been shown to have several biological activities such as antidiabetic, anticough, antioxidant, antibacterial and antispasmotic properties [6]. Most of the pharmacological and chemical work has been carried out on the leaf, since the leaf of the guava is rich in flavonoids and phenols including terpenoids, tannins, essential oils, chlorophyll and saponins [7]. The aqueous and alcoholic extracts of guava (root as well as leaves) were found to have inhibitory effects on the growth of *Staphylococcus aureus, Streptococcus mutans, Pseudomonas auruginosa, Salmonella enteritidis, Bacillus cerus, Proteus* spp., *Shigella* spp., and *Escherichia coli,* causal agent of intestinal infections in humans. These effects were examined using the in vitro agar well diffusion method [8]. In another study, aqueous and methanolic extracts of the leaves are effective inhibitors of growth spore formation and enterotoxin production of *Clostridium perfringens* type A [9]. Its antimicrobial activity was beneficial when applied as an antimicrobial agent in textiles.

Microencapsulation technology is a well-known technique for finishing textiles. This technique is based on active compounds being encapsulated using a material wall for long-acting release and protection from the environment [10]. Encapsulation in a poly(urea-formaldehyde) shell (PUF) has proven to be one of the most versatile and widely used encapsulation approaches. Microcapsules (MCs) are produced by the polymerization and deposition of the UF polymer at the interface of the suspended emulsion droplet. Polymerization of the shell wall occurs in the aqueous phase until a critical molecular weight is achieved and the polymer phase separates and is deposited at the encapsulent-aqueous interface [11]. The deposited polymer forms the shell wall of the microcapsule. The increase in molecular weight of the urea-formaldehyde resin occurs under acidic conditions [12]. Ammonium chloride and ammonium sulfate are the most widely used catalysts and resorcinol is used as a cross-linking agent.

Cotton is widely used as a textile material, as it is soft and comfortable to wear. However, its porous hydrophilic structures retain water, oxygen, and nutrients, which provides a perfect environment for the growth of microorganisms [14]. N. T. Hein, S. S. Hnin, and D. H. Htay studied the effect of antimicrobial agents from Aloe Vera gel on bleached cotton fabric. This study revealed that the antimicrobial activity of Aloe Vera gel treated fabric was excellent for inhibiting *Pseudo* and *E. coli* and good for inhibiting *B. aureus* and *B. pumilus* bacteria, but it could not inhibit the growth of *S. aureus* and *Candi* [15]. S. Sharaf, A. Higazy, and A. Hebeish

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Jiraphorn Katewaraphorn and Arunee Kongdee Aldred are with the Applied Chemistry Program, Faculty of Science Maejo University, 50290 Thailand (e-mail: aonoe27@hotmail.com, akongdee@hotmail.com).

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applied propolis extract with glyoxal and $Al_2(SO_4)_3$ catalyst using the pad-dry method to produce a cotton textile with superior antibacterial activity, water repellant properties, and UV protection [16]. Plant extracts from the *Jatropha Curcas* leaf were used to treat cotton fabrics by the direct application method and antimicrobial activity was determined by an agar plate test. The results showed that the plant extract demonstrated a considerable zone of inhibition to *S. aureus* and acted as a bactericide [17]. The extract of pomegranate (*Punica granatum*) was used for dyeing cotton fabric. The antimicrobial activity was assessed qualitatively by the disc diffusion method and AATCC 147, and was quantitatively tested by AATCC 100 against *E. coli* and *S. aureus*. Pomegranate extract displayed excellent antibacterial activity against both of the test organisms [18]. Moreover, acetic acid soluble material was isolated from the cell wall of *Mucor rouxii* DSM-1191 and applied on cotton fabrics. The results showed that *Mucor rouxii* DSM-1191 has excellent antibacterial activity against *E. coli* and *M. luteus* [19].

The aim of this work was the encapsulation of guava leaf extracts into urea-formaldehyde microcapsules by in situ polymerization, in order to develop an eco-friendly natural antimicrobial finish in textiles. Cotton was finished with an antibacterial agent extracted from the guava leaf in this work. In addition, the finished fabric was assessed for antibacterial activity using AATCC 147-2004 against test organisms of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). *S. aureus* is a major disease-causing bacteria that exists in the armpit, inner elbow, between the mid-buttocks, the sides of the groin, and the bottom of the head. *E. coli* is also considered a potential pathogen and resides mainly in the small intestine. However, it can also be found in the inguinal and perineal areas contaminated by urine and feces [20].

II. Procedure

A. Materials

Urea, 36% formaldehyde, and acetic acid were purchased from Prolab. Ammonium acetate and Folin-Ciocalteu reagent were purchased from QRëC®. Polyvinyl alcohol (PVA) and zinc nirate were provided by Ajax Finechem PTY. Ammonium chloride was obtained from Rankem, and Resorcinol from HIMEDIA Labaratories Pvt. Helizarin, Fixapret F-Eco and Condensol were obtained from BASF. Triplicate soy gar and Triplicate soy broth were provided by Difco, and Acetylacetone by ACROS. Carboxymethyl cellulose (CMC) was obtained from Sigma Aldrich.

B. Preparation of Guava Leaf Extract

The fresh leaves of *Psidium guajava* L. were collected from Chiang Mai. Then, the leaves were washed to remove debris and dried at 50°C for 24 hours in an oven. After being completely dried, the leaves were crushed into small pieces. Samples were extracted with distilled water in a ration of 1:15 at 60°C for 1 hour. The extracts were sieved through cheesecloth followed with Whatman No.3 filter paper. The filtrates were concentrated by water evaporation and freeze-dried at -20°C. Crude extracts were stored at 4°C for further application.

C. Preparation of MCs Containing Guava Leaf Extract

PUF MCs containing guava leaf extract were prepared by in situ polymerization according to the procedure of Suryanarayana [21] with modifications. Firstly, 5 g of urea, 0.5 g ammonium chloride and 0.5 g resorcinol were dissolved in 260 ml distilled water. Secondly, 10 ml of 5% wt aqueous solution of PVA was added into the solution and the pH of the solution was adjusted to 3.5 with 5% hydrochloric acid. After 5 g extract was loaded into the solution for 10 min, 12 ml of 36% wt formaldehyde solution was added. The reaction was constantly stirred at 800 rpm and heated at 55°C for 4 hours. The obtained MCs were subsequently filtered with Whatman No.3 paper under a vacuum and washed with distilled water. Finally, the prepared MCs were dried at room temperature and stored under a vacuum condition.

D. Characterization of Microcapsules

The morphology of the MCs was observed using a scanning electron microscope (SEM, 5410LV JEOL). The MC samples were dispersed in distilled water and sonicated for 30 min. After that, one drop of the MC dispersion was placed on the surface of a double-faced black adhesive tape attached to a stainless steel stub and dried at 50°C for 1 hour. The samples were sputtered with a thin layer of gold.

The MCs containing guava leaf extract were analyzed using a Fourier-transform infrared spectrometer (FTIR, Perkin Elmer) to identify the chemical structure of the samples. The samples mixed with potassium bromide (KBr) were ground and analyzed in KBr pellet form. The size of the MCs was also analyzed using a particle size analyzer (Mastersizer, MALVERN).

E. Finishing of Cotton Fabric

Both the guava leaf extract and the MCs containing guava leaf extract were applied to cotton fabric using the printing method. 50 g/l of guava leaf extract/MCs was mixed with 2% wt of CMC, 50 g/l of Helizarin, 50 g/l of Fixapret F-Eco, 20 g/l of Condensol and 10 g/l of zinc nitrate. The cotton fabric was coated with the mixture using a printing machine (Kidd + Zigrino). The fabric was dried at 105°C for 90 seconds and cured at 175°C for 45 seconds. The fabric was washed with water before further testing. The modified surface of the finished fabric was observed using SEM.

F. Antibacterial Activity Testing

The finished fabric was assessed for antibacterial activity according to modified AATCC147-2004 [22]. The bacteria species of Gram positive *Eschericia coli* and Gram negative *Stapphylococcus aureus* were used in this standard. The cell concentration of bacteria for this work was 10^8 CFU/ml. Nutrient agar plates were streaked with 5 lines of bacteria from an inoculation loop. Then, the fabric samples were placed over the incubated agar culture. After incubation at 37°C for 18-24 hours, the inhibition zone was observed.

G. Determination of Formaldehyde Content

The method for determination of free formaldehyde was the 2,4-pentanedion method according to BS EN ISO 14184-1:1999 part 1 Annex A [23]. Firstly, a 0.5 g fabric sample was put into a 250 ml flask and followed with 20 ml of deionized water. The flask was covered with a stopper and

heated in a water bath at 40°C for 1 hour with a laboratory shaker. Then, the warm solution was filtered into another flask through a Whatman filter. After 5 ml of solution sample was transferred into a tube, 5 ml of acetylacetone solution was added into the tube, which was covered with a stopper and shaken. The mixture was kept first in a water bath at 40°C for 30 min and then was left at room temperature for 30 min. Blank reagent was prepared by adding 5 ml of acetylacetone solution to 5 ml deionized water and treating it the same way. The absorbance was measured at 415 nm using a spectrophotometer (U2001 Hitashi, Japan). Formaldehyde solution (0.15-6.00 μg/ml) was prepared as the calibration solution. Each test was performed in triplicate.

III. Results and Discussion

A. Characterization of MCs

First of all, encapsulation of guava leaf extract in a PUF shell is carried out when the pH becomes acidic, is heated to 55°C, and reacts with urea and formaldehyde resulting in a PUF. In the initial step of polymerization, the urea-formaldehyde molecule is rich in polar groups and is water compatible. The product of this step is called methyl urea. Next, the number of polar groups is gradually reduced as the molecular weight of the polymer increases. Finally, the hydrophilicity of the PUF molecule is reduced leading to separation from the aqueous phase, and droplets of MC powder are received [24]. MC powder without extract is white, whereas MC powder containing extract is brown.

The structure of PUF MCs was investigated using FTIR to confirm the existing core material and guava leaf extract. The FTIR spectrum of the guava leaf extract, PUF shell, and PUF MCs containing guava leaf extract are presented in Fig. 1. The spectra of the PUF shell and PUF MCs containing guava leaf extract show a similar pattern: peaks of C=O stretching vibration at 1650 cm^{-1}, N=H stretching vibration at 1554 cm^{-1}, and C-N stretching vibration at 1245 cm^{-1}. These spectrums confirm the formation of the PUF wall of the MCs. Furthermore, the presence of peaks at 1444 cm^{-1} and 1118 cm^{-1} in the spectrum band of PUF MCs containing guava leaf extract correspond to the C-H stretching vibration and C-C stretching vibration in the spectrum of guava leaf extract. Along with the O-H peak at 3700-3000 cm^{-1}, it shows a broader band which corresponds to the –OH group in phenolic compounds. It shows that the guava leaf extract is successfully encapsulated in the PUF shell.

Fig. 2 illustrates SEM images of MCs without extracts and MCs containing guava leaf extract. MCs without extract have a spherical particle and smooth outer surface. When the extract was loaded into the PUF, the MCs are larger and have an irregular shape. Additionally, the MCs tend to agglomerate to form a large mass of small particles.

Fig. 3 reveals the size distribution of MCs. The size of MCs without extract is distributed into 2 ranges: a large group of 8-50 micrometers and a smaller group of 50-140 micrometers. The diameter of MCs containing extract was larger and ranged from 40-180 micrometers. This may be caused by encapsulation of the extract by PUF.

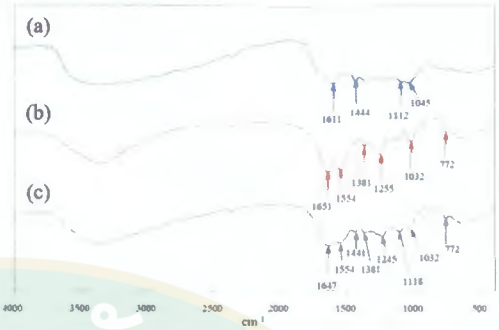

Fig. 1. FTIR spectra of (a) guava leaf extract (b) PUF shell (c) PUF MCs containing guava leaf extract.

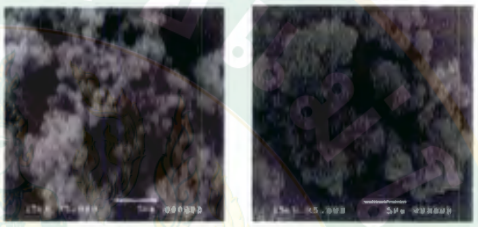

Fig. 2. SEM images of (a) PUF MCs without extract; (b) PUF MCs containing guava leaf extract.

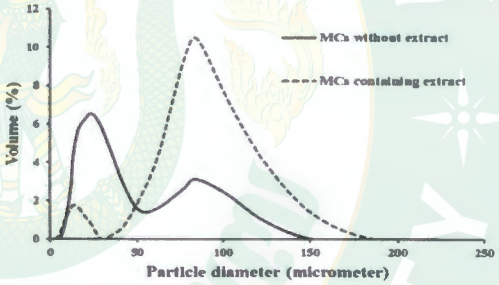

Fig. 3. Particle size distribution of MCs.

B. Characterization of Finished Fabric

Fig. 4 shows cotton fabric finished with guava leaf extract and MCs containing guava leaf extract by the printing method. The fabric finished with guava leaf extract has a more intense homogeneous brown color and a smoother surface than fabric finished with MCs containing guava leaf extract. For cotton fabric finished with MCs containing guava leaf extract, MCs are deposited on the fabric surface with the assistance of Helizarin and Fixapret F-Eco.

C. Assessment of Antibacterial Activity

The finished fabrics were assessed for antibacterial activity against *E. coli* and *S. aureus* using AATCC147-2004. The results are shown in Fig. 5 and Fig. 6. There is no zone of inhibition in any of the fabrics against *E. coli*. The unfinished cotton fabric is shown by (b), cotton fabric finished with guava leaf extract is (a), and cotton fabric finished with MCs containing guava leaf extract is (c). However, there is no growth of *E. coli* underneath the fabric finished with guava leaf extract and the fabric finished with MCs containing guava leaf extract.

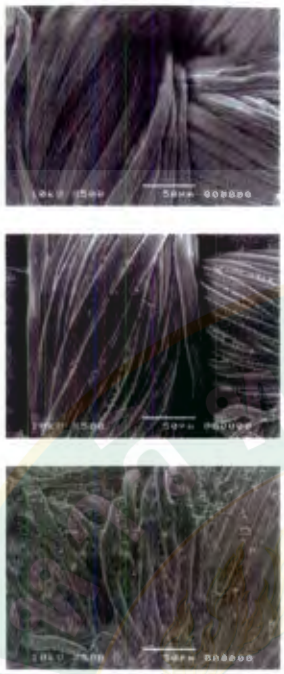

Fig. 4. SEM images of (a) unfinished cotton fabric; (b) cotton fabric finished with guava leaf extract (c) cotton fabric finished with MCs containing guava leaf extract.

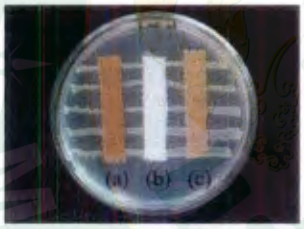

Fig. 5 Antibacterial activity results against *E.coli* of (a) cotton fabric finished with guava leaf extract; (b) unfinished cotton fabric; (c) cotton fabric finished with MCs containing guava leaf extract.

Fig. 6. Antibacterial activity results against *S.aureus* of (a) cotton fabric finished with guava leaf extract; (b) unfinished cotton fabric; (c) cotton fabric finished with MCs containing guava leaf extract.

Fig. 6 shows the result of antibacterial activity against *S. aureus* . The fabric finished with MCs containing guava leaf extract © has an obviously larger zone of inhibition that that of the fabric finished with guava leaf extract (a). The results show that fabric finished with MCs containing guava leaf extract shows good antibacterial activity against *S. aureus*. Although the fabric finished with guava leaf extract has no inhibition zone, *S. aureus* cannot grow underneath the fabric. This result indicates that cotton fabric finished with MCs containing guava leaf extract can inhibit *S. aureus* better than *E. coli*.

D. Effect of Formaldehyde Amount

Both the fabric finished with extract and the fabric finished with MCs were analyzed for formaldehyde residue according to the BS EN ISO 14184-1:1999 standard. The results showed that the free formaldehyde amount on the fabric finished with extract and MCs was 0.30 and 1.51 ppm/g of fabric. However, the weight of each sample fabric for antibacterial testing was approximately 0.4 g. Therefore, it can be assumed that the fabric samples contained 0.12 and 0.60 ppm formaldehyde for the cotton fabric finished with guava leaf extract and the cotton fabric finished with MCs containing guava leaf extract, respectively. Thus, it is likely that the amount of formaldehyde did not affect the inhibition of organism growth.

IV. Conclusion

MCs containing guava leaf extract were successfully constructed by in situ polymerization of PUF shells under acidic conditions. The surface morphology of MCs containing guava leaf extract was rough with irregular particles. The formation of MCs confirmed the encapsulation of guava leaf extract in the PUF shells by the intensity of -OH group in the phenolic compound at 3700-3000 cm^{-1} and the absorption bands at 1444 cm^{-1} and 1118 cm^{-1} which corresponded with the bands in the spectra of guava leaf extract. Finally, cotton fabric finished with MCs containing guava leaf extract showed antibacterial activity against *S. aureus*, but it was not effective against *E. coli*. It can be concluded that guava leaf extract MCs are effective for application as an antibacterial agent for finishing textiles. However, more research needs to be done on wash durability testing and quantitative testing of antibacterial activity.

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Jiraphorn Katewaraphorn was born in Chumporn, Thailand, in 1982. She received a B.S. degree in chemistry from Naresuan University, Phitsanulok, Thailand, 2004 and an M.S. degree in biotechnology from Chiang Mai University, Chiang Mai, Thailand, in 2010.

She is currently a Ph.D. student at Maejo University, Chiang Mai, Thailand. Her major is applied chemistry and her research interest is the encapsulation of natural products and finishing textiles. She published the title of Microencapsulation of *Psidium guajava* Linn. Leaf Extract in 2013 and Preparation of Microcapsules Containing *Sapindus rarak* DC. Extract in 2014. In 2007, she worked as an R&D chemist at Winson Screen Co., Ltd. In 2010, she joined the Program of Applied Chemistry, Faculty of Science and Technology, Rajabhat University, Uttaradit, Thailand, as a lecturer.