การพัฒนาอนุภาคขนาดเล็กต้านจุลินทรีย์เพื่อใช้ในอาหารที่ไม่ผ่านการฆ่าเชื้อเชิงพาณิชย์

นางสาว ปาจรีย์ ตั้งศิริวัฒนา

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DEVELOPMENT OF ANTIMICROBIAL MICROPARTICLE FOR USE IN COMMERCIALLY NON-STERILIZED FOOD

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Field of Study	Biotechnology
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ปาจรีย์ ตั้งศิริวัฒนา : การพัฒนาอนุภาคขนาดเล็กต้านจุลินทรีย์เพื่อใช้ในอาหารที่ไม่ผ่านการฆ่าเชื้อเชิงพาณิชย์. (DEVELOPMENT OF ANTIMICROBIAL MICROPARTICLE FOR USE IN COMMERCIALLY NON-STERILIZED FOOD) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.จิรารัตน์ ทัตติยกุล อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ. ดร. ชื่นจิต ประกิตชัยวัฒนา 140 หน้า.

น้ำมันกระทียมมีสารออกฤทธิ์ทางชีวภาพซึ่งอยู่ในกลุ่มสารประกอบซัลเฟอร์หลายชนิด มีฤทธิ์ในการยับยั้งจุลินทรีย์ทั้งแกรมบวกและแกรมลบ แต่มีความคงตัวต่ำ งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อพัฒนาอนุภาคไมโครแคปซูลสารต้านจุลินทรีย์จากน้ำมันกระทียมโดยมีมอลโตเดกซ์ตริน (DE=10) เป็นตัวพาและใช้เทคนิคการอบแห้งแบบพ่นกระจาย เพื่อนำไปประยุกต์ใช้ในน้ำสลัดต้นแบบ ซึ่งเป็นอาหารพร้อมบริโภคเชิงพาณิชย์ที่ไม่ได้ผ่านการฆ่าเชื้อด้วยความร้อน งานวิจัยนี้แบ่งออกเป็น 3 ส่วนหลัก ส่วนที่หนึ่งเป็นการศึกษาสัดส่วนที่เหมาะสมของอิมัลชันปฐมภูมิสำหรับพ่นแห้ง โดยเริ่มจากการประเมินค่าความเข้มข้นวิกฤตของการเกิดไมเซล (CMC) ของสารละลายโพลีซอร์เบท (Tween®) 20 และ Tween®80 ในสารละลายมอลโตเดกซ์ตรินเข้มข้น 20 กรัมต่อเดซิลิตร พบว่าทั้ง Tween®20 และ Tween®80 มีค่า CMC เท่ากับ 0.49 เปอร์เซ็นต์โดยน้ำหนัก จึงเลือกใช้ Tween®20 เป็นสารทำอิมัลชัน จากนั้นเป็นการศึกษาสภาวะที่เหมาะสมในการเตรียมอิมัลชันปฐมภูมิเตรียมอิมัลชันโดยแปรความเข้มข้นของ Tween®20 เป็นร้อยละ 0.6 0.8 และ 1 ในสารละลายมอลโตเดกซ์ตริน 20 กรัมต่อเดซิลิตร แปรอัตราส่วนระหว่างน้ำมันกระเทียมและมอลโตเดกซ์ตรินเท่ากับ 0.1:1 0.15:1 และ 0.2:1 และแปรภาวะในการโฮโมจิไนซ์ โดยแปรความเร็วรอบเป็น 13,000 19,000 และ 24,000 รอบต่อนาที เป็นระยะเวลา 5 และ 10 นาที พบว่า อิมัลชันที่เตรียมได้ในทุกภาวะมีความคงตัวดี ไม่แยกชั้นเมื่อเก็บที่อุณหภูมิห้อง (~25 องศาเซลเซียส) เป็นเวลา 48 ชั่วโมง และมีหยดน้ำมันขนาดเล็กในช่วง 0.126 ถึง 0.281 ไมครอน โดยที่ภาวะสัดส่วนน้ำมันต่อมอลโตเดกซ์ตริน 0.2:1 ความเร็วรอบโฮโมจิไนซ์ 19,000 รอบต่อนาที เป็นระยะเวลา 5 นาที ให้อิมัลชันที่มีความคงตัวดี หยดน้ำมันมีขนาดเล็ก (0.124 ถึง 0.216 ไมครอน) ประสิทธิภาพในการเอนแคปซูเลทน้ำมันดี (~18.45%) จึงเลือกภาวะนี้ในการเตรียมอิมัลชันปฐมภูมิสำหรับการพ่นแห้ง ส่วนที่สองเป็นการศึกษาสภาวะที่เหมาะสมในการเตรียมไมโครแคปซูลน้ำมันกระทียมโดยการอบแห้งแบบพ่นกระจายโดยแปรอุณหภูมิขาเข้าเป็น 4 ระดับคือ 120 160 180 และ 200 องศาเซลเซียส กำหนดอัตราพ่น 25 มิลลิลิตรต่อนาทีที่ความดัน 3 บาร์ พบว่าไมโครแคปซูลมีลักษณะกลมหรือค่อนข้างกลม มีขนาดเฉลี่ย D[3,2] อยู่ในช่วง 9 ถึง 13 ไมครอน มีรูพรุนกระจายอยู่บนผิวของอนุภาค ผิวเรียบ ขณะที่อนุภาคที่เตรียมที่อุณหภูมิขาเข้า 120 องศาเซลเซียส มีพื้นผิวขรุขระ มีหลุมกระจายบนพื้นผิว และมีค่าความหนาแน่นรวมสูง โดยอุณหภูมิขาเข้าในระดับที่สูงขึ้นยังส่งผลให้ไมโครแคปซูลมีความชื้นและค่าแอกติวิตีของน้ำแนวโน้มลดลง แต่ค่าการละลายไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ปริมาณน้ำมันที่ห่อหุ้มได้อยู่ในช่วงร้อยละ 1.34 ถึง 3.69 พบสารไดอะริล โมโน, ได-, ไตร- ซัลไฟด์ปริมาณต่ำ ไมโครแคปซูลสามารถยับยั้ง *Staphylococcus aureus* โดยมีค่าความเข้มข้นต่ำสุดในการยับยั้ง (MIC) เท่ากับ 0.1 กรัมต่อกรัม แต่ไม่พบการยับยั้งใน *Salmonella* Typhimurium และ *Escherichia coli* เมื่อประเมินการปลดปล่อยสารของไมโครแคปซูลที่เตรียมจาก Tween®20 0.6% ที่อุณหภูมิการพ่นแห้ง 180 องศาเซลเซียส พบว่ามีการปลดปล่อยออกมาทันที (burst release) เมื่อเติมอนุภาคในน้ำสลัดพบว่าช่วยชะลอการเสื่อมเสียได้ 1 วัน ที่ 25 องศาเซลเซียส โดยสามารถลดจุลินทรีย์ทั้งหมดได้ 1.4 log CFU/g ในวันที่ 2 การเติมอนุภาคมีผลทำให้ค่าความสว่าง (L^*) และสีแดง (a^*) ของน้ำสลัดเพิ่มขึ้น สีเหลือง (b^*) ลดลง จากผลการทดสอบทางประสาทสัมผัสโดยใช้ผู้ทดสอบ 50 คน ระหว่างการเก็บเป็นเวลา 5 วัน ผู้ทดสอบประเมินว่าน้ำสลัดที่เติมไมโครแคปซูลมีสีเข้มขึ้นความเรียบเนียนและความหนืดลดลงเล็กน้อย

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต.....ปาจรีย์ ตั้งศิริวัฒนา.....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5072355023 : MAJOR BIOTECHNOLOGY

KEYWORDS : GARLIC OIL / MICROENCAPSULATION / SPRAY DRYING / ANTIMICROBIAL

PAJAREE TANGSIRIWATTANA : DEVELOPMENT OF ANTIMICROBIAL MICROPARTICLE FOR USE IN COMMERCIALLY NON-STERILIZED FOOD. ADVISOR : Assistant Professor Jirarat Tattiyakul, Ph.D., CO-ADVISOR : Assistant Professor Cheunjit Prakitchaiwattana, Ph.D., 140 pp.

Garlic oil contains bioactive components that are sulfide derivatives, which possess antimicrobial activity against various gram-negative and gram-positive bacteria. However, the oil is volatile and heat sensitive. The objective of this research was to develop antimicrobial microparticle of garlic oil using maltodextrin (DE 10) as carrier by spray drying technique, in order to apply in salad dressing that is not commercially sterilized and packed under presence of oxygen. This research is divided in to 3 parts. Firstly, the optimal proportion of initial feed emulsion was determined. Determination of critical micelle concentration (CMC) of polysorbate (Tween®) 20 and Tween®80 in 20 g/dL maltodextrin was carried out. It was found that CMC of Tween®20 and Tween®80 was 0.49% w/w. Thus, Tween®20 was selected as the emulsifier for subsequent studies. For determination of optimal emulsion preparation, emulsions containing Tween®20 at various concentrations (0.6%, 0.8% and 1% w/w) in 20 g/dL maltodextrin and 0.1:1 0.15:1 and 0.2:1 oil-to-maltodextrin ratio were prepared. Homogenization was carried out at 13,000, 19,000 and 24,000 rpm for 5 and 10 min. It was observed that all prepared emulsions were stable at room temperature (~25°C) for 48 hours. Oil droplet size ranged from 0.126 to 0.281 μm. The emulsion containing 0.2:1 oil-to-maltodextrin ratio homogenized at 19,000 rpm for 5 min had good stability, contained smaller oil droplet size (0.124 to 0.216 μm), and gave rise to higher encapsulation efficiency (18.45% oil retention). This condition was thus chosen for preparing the emulsion feed. In the second part, the optimal condition for spray drying was investigated. The inlet air temperature was varied from 120°C to 200°C, and the initial feed was spray-dried at 25 mL/min at 3 bars. Most microcapsules were spherical with small holes dispersing on their surface. The microcapsules had the surface average diameter; D[3,2], from 9 to 13 μm and had smooth surface, except those spray-dried at 120°C that had rough surface. Moisture content and water activity of microcapsules decreased with increasing the inlet air temperature. The solubility was not significantly different ($p>0.05$). The microcapsules contained 1.34 to 3.69% total oil. They contained di- and tri- sulfides from 0 to 0.05 and 0 to 0.01 mg/g, respectively. The microcapsules could inhibit the growth of *Staphylococcus aureus* at minimum inhibitory concentration (MIC) of 0.1 g/g, but showed no inhibition against *Salmonella* Typhimurium and *Escherichia coli*. The garlic oil release from the microcapsules prepared from 0.6% w/w Tween®20 and 0.2:1 oil-to-maltodextrin ratio spray-dried at 180°C was of the burst release characteristic. The addition of garlic oil microcapsules could extend the storage life of salad dressing for up to 1 day at 25°C and reduced 1.4 log CFU/g total bacteria at day 2. The salad dressing containing garlic oil microcapsules had higher L* and a* than the control sample. From the sensory assessment by 50 assessors, the salad dressing containing the microcapsules was darker, had higher smoothness and lower viscosity.

Field of Study : BIOTECHNOLOGY Student's Signature Pajaree Tangsiriwattana

Academic Year : 2009 Advisor's Signature

Co-Advisor's Signature Cheunjit

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CHAPTER I

INTRODUCTION

Salad dressing is one of the non-sterilized ready-to-eat foods, which are susceptible to growth of food pathogens, e.g. *Salmonella* spp., *S. aureus* and *E. coli* when it is not refrigerated (Erikson, 1991; Snyder, 1998). In general practice, chemical preservatives are used to inhibit food pathogens and spoilage microorganisms. Owing to rising concern on the use of chemical substances in food product of health conscious consumers, alternative natural preservatives have been proposed.

Garlic (*Allium sativum* Linn) is an herbal plant widely grown in Thailand. Garlic is a food item which can potentially benefit human health. It has been reported to possess antimicrobial activities (Cavallito et al., 1944; Rees, 1993). The most significant bioactive components of garlic, are organosulfur-containing compounds (11-35 mg/g fresh garlic) (Nagpurkar et al., 2000), for example, thiosulfinates, volatile odor producing substances formed enzymatically when garlic is crushed. The main antimicrobial effect of garlic is due to allicin (allyl 2-propene-thiosulfinate), a thiosulfinate formed via allinase reaction, which reacts with thiol group of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase (Ankri et. al., 1997; Wills, 1956). Many researchers have been interested in using garlic as an antimicrobial agent (Kim, 2000; Krest et al., 2000; Ross et al., 2001). Althought allicin has antimicrobial potency, it is unstable at high temperature (Siripongvutikorn et al., 2005). Garlic oil is both volatile and hydrophobic. When it is subjected to heat, allicin is transformed into more stable derivative compounds that may possess reduced antimicrobial activity. To overcome this problem, microencapsulation of garlic oil is necessary.

Microencapsulation involves the process of encapsulating or coating ingredients into a protective film of another material. The most general technique for microencapsulating sensitive ingredients, especially volatile and essential oil, is by using a spray dryer. Owing to short contact time between hot air and the droplets, loss of activity of bioactive ingredient is minimal. Several studies have also looked at the possibility of using this process for the preservation of probiotic bacteria (Silva et al.,

2002). The results of their experiments showed high survivor rates of microorganisms at low storage temperature.

This research aims to develop garlic oil antimicrobial microparticles for application in commercial non-sterilized food. Salad dressing was used as a food model in this study. Firstly, the optimum condition of preparing emulsions for subsequent studies was investigated. Secondly, the optimum spray-dried condition to produce desired microparticles was determined. Thirdly, the properties of microcapsules, i.e., physical and chemical properties, sensory assessment, and antimicrobial assay, were observed.

CHAPTER II
LITERATURE REVIEW

2.1 Garlic

Garlic (*Allium sativum* Linn.) has been widely used as a food ingredient and a medicinal agent for centuries. It is noted "Generally Regarded as Safe" (GRAS) by the United States Food and Drug Administration (USFDA). Garlic is an herbal plant widely grown in Thailand. It appears to be a food item which can potentially benefit human health (Fenwick and Hanley, 1985; Nagpurkar et al., 2000).

In recent years, there are several papers about garlic or its constituents. A number of studies have shown an inhibitory effect of fresh and freeze-dried garlic extracts on many microorganisms. For instance, Cavallito and coworkers (1945) reported the antibacterial properties of garlic clove homogenates against *Escherichia-coli* and *Staphylococcus aureus*. Yet the complex biological actions of them are still not completely understood.

Garlic is composed mainly of water (56-68%) and the most significant components, bioactive agent, are the organosufur-containing compounds (11-35 mg/g fresh garlic) (Lawson, 1996). Garlic clove contains mainly cysteine sulfoxide such as alliin (5-10 mg/g fresh garlic) followed by methin and isoallin that are formed from γ-glutamyl-cysteines (γ-glutamyl-S-*trans*-1-propenylcysteine, γ-glutamyl-S-allylcysteine, and γ-glutamyl-S-methylcysteine). When garlic is crushed, enzyme allinase or alliin lyase is released to convert cysteine sulfoxides into thiosulfinates (that contains 60-80 % allicin) (Prasad et al., 1996; Rabinkov et al., 1998; Li and Xu, 2007), which are reactive, volatile, odor producing substances. Allicin (allyl-2-propenethiosulfinate) is the most abundant thiosulfinate (approximately 70%) formed via allinase reaction (Figure 2.1). The stability of thiosulfinates depends on solvent, temperature, concentration, and purity. Half-life of pure allicin in water and 1 mM citric acid is 30 and 60 days, respectively, while that of allicin without a solvent decreases to 16 hours (Lawson, 1993).

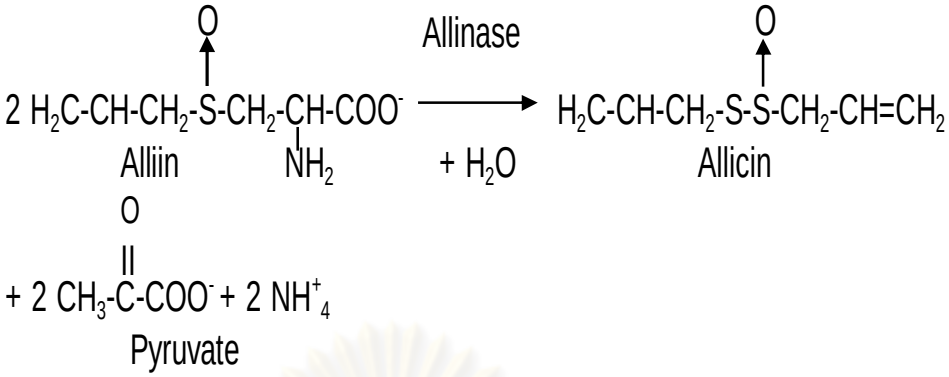


Figure 2.1 Generation of allicin through the allinase reaction.

Source: Ankri and mirelman (1999)

Since 1994, after allicin was identified, many researches have been focused on the thiosulfinate of garlic.

Fresh garlic and garlic powders have been prepared by carefully drying at low temperatures (<60˚C) to keep the capability of producing bioactive allicin. Some products such as garlic macerates in oil and garlic oil products do not only generate allicin but also contain allicin-derived compounds. These products contain varying amount of allicin-derived compounds like methyl and allyl sulfide derivative (Lawson, 1996; Ross, 2001). In recent years, garlic products have been popular as health foods with beneficial physiological effect for human. Thus, there are many researches investigating the application of garlic in new products. Several types of garlic products are prepared under a variety of conditions such as low temperature drying, steam distillation and long-term incubation in various media (Table 2.1) (Nagpurkar et al., 2001).

Table 2.1 Commercially available products and their possible active principles

Product	Processing	Possible active principle(s)	Note
Fresh garlic	None	Allicin and allicin derivative compound generated *in vivo*	Heating may cause loss of allinase activity
Garlic powder tablet	Drying/grinding	Allicin and allicin derivative compound generated *in vivo*	May be enteric or non enteric coated and/or standardized for allin content
Oil mercerated garlic	Incubation in oil	Vinyl dithiins, ajoenes, allyl sulfides	Not often found commercially in North America
Garlic oil	Steam distillation	allyl di-, trisulfides	-
Aged garlic extract	Incubation in ethanol	S-allylcysteine (SAC) S-allylmercaptocysteine	Contains no allicin or allicin-derived compound

Source: Nagpurkar et al. (2000)

Methyl and allyl sulfide derivatives of allicin are formed by steam distillation of chewed garlic to produce garlic oil (GO) (Lawson, 1996) used in medicinal products. Although some early studies concluded that GO has low antimicrobial activity (Adetumbi et al., 1986; Chung et al., 1998), recent studies revealed that GO and its major diallyl sulfides, i.e. diallyl disulfides (DADS), diallyl trisulfides (DATS) possess antimicrobial activity against various gram-negative and gram-positive bacteria (O'Gara et al., 2000). Recently, Ross et. al. (2001) showed that GO in which the most abundant constituents consist of DADS and DATS, revealed broad-spectrum antimicrobial activities, especially

against several pathogenic species which cause food poisoning. The minimum inhibitory concentration (MIC) results for GO over 24 and 48 hours from their study are shown in Table 2.2. Moreover, Han et al. (1995) found that the antibiotic activity of 1 mg of allicin is equal to that of 15 IU (International unit) of penicillin (Han et al., 1995).

Mechanism of allicin action is specific inhibition on other bacterial enzymes such as the acetyl-CoA-forming system (acetyl-CoA synthetase or acetyl-CoA ligase, E.C.6.2.1.1) consisting of acetate kinase and phosphotransacetyl-CoA synthetase by means of the thiol disulphide exchange reaction of allicin with free thiol group in proteins or SH-containg enzyme that results in loss of protein function (Figure 2.2) (Focke et al., 1990; Wills, 1956). The inhibition is non-covalent and irreversible.

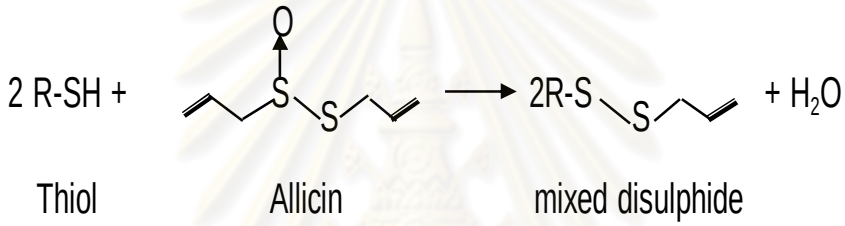


Figure 2.2 The generic thiol disulphide exchange reaction between allicin and thiol.

Source: Curtis et al. (2004)

Many studies developed various types of garlic products for use and handling in order to preserve the biological activity of allicin. Although freeze-drying (FD) is a convenient process that yields dehydrated garlic with an excellent quality, it is one of the most expensive processes owing to large capital outlays and high operating cost. Therefore, other processes have been investigated for the successful production of garlic products (Li and Xu, 2007).

Recently, Li and Xu (2007) studied the properties of garlic powder which was microencapsulated by microwave-vacuum drying (MVD) to prolong shelf life and protect allinase activity. They concluded that garlic powder prepared by MVD technology could also provide high allicin content of 90.2% and the quality of the final product was as good as the product prepared by freeze drying method.

Table 2.2 Minimum inhibitory concentration of garlic oil against human enteric pathogens[a]

Bacterium	No. of strains tested	MIC (mg/ml)	
		24 h*	48 h*
Human enteric pathogens			
Bacillus cereus	1	0.08	0.17
Escherichia coli O55	1	5.5	5.5
Escherichia coli O128	1	2.75	2.75
Escherichia coli O112	1	2.75	5.5
Shigella boydii	1	1.37	2.75
Shigella flexneri	1	1.37	2.75
Shigella sonnei	4	2.75	2.75–5.5
Vibrio fluvialis	1	2.75	2.75
Vibrio metschnikovii	1	0.02	0.34
Vibrio parahaemolyticus	1	0.04	0.08
Yersinia enterocolitica	3	0.17–0.34	0.68
Listeria monocytogenes	2	0.02	0.02–0.08
Salmonella enterica serovar Enteritidis	2	5.5	5.5
Salmonella enterica serovar Infantis	1	2.75	5.5
Salmonella enterica serovar Senftenberg	2	5.5	5.5
Salmonella enterica serovar Typhimurium	3	0.34–2.75	0.68–5.5
Campylobacter jejuni	4	0.16–0.32	0.16–0.32
Campylobacter coli	1	0.16	0.49
Campylobacter lari	1	0.16	0.49

[a] Each MIC determination was performed in triplicate per bacterial isolate

* Incubation time

Source: Ross et al. (2001)

2.2 Foodborne Illness from Salad Dressing

Dressed salad, non-sterilized food, is a mixture of various foods. The main components of salad dressing contain raw egg, sugar, spices and organic acids. Because of the addition of vinegar, salad dressing typically has a pH between 4.0 and 5.5. The acetic level in the aqueous phase is much lower than that in mayonnaise, and is often between 0.2% and 0.5%. Commercial salad dressing is not sterilized by treatment at high temperature because it would destroy the physical integrity and directly affect sensory qualities substantially. Therefore, dressed salads should be kept refrigerated to extend their shelf life. The chilled shelf life of commercial salad dressing products is typically between 2 and 8 weeks (Lund et al., 2000). The initial microflora of salad dressing is made up of the microbial load of the raw materials. The incorporation of raw materials can also lead to the contamination of pathogen resulting in foodborne illnesses. Pathogens such as *Escherichia coli* O157:H7 (acid-tolerant), *Salmonella* spp., and *Staphylococcus aureus* are significant source of illness (Table 2.3). Growth of pathogens represents an evident safety hazard for salads with a high pH or final pH values above 4.1, when salads are not kept at refrigerated temperatures. At 22˚C to 32˚C, *Salmonella* and *Staphylococcus aureus* could grow well within 24 hours but no growth occurred at 4˚C (Lund et al., 2000). Several salmonellosis outbreaks were caused by mayonnaise and salad dressing. For instance, in the United States, 404 of 965 persons in New York hospital became ill and 9 people died. The source of the incident was hospital-prepared mayonnaise made with raw egg contaminated with *S. enteritidis* (Telzak et al., 1990). In 1992, 81 guests and 11 catering staffs became ill at a wedding reception due to *S. enteritidis* from mayonnaise (Chandrakumer, 1995). When citric acid and low level of acetic acid is used as an acidulant in mayonnaise (~pH 5.0), *S. enteritidis* can survive for a few days at 20ºC or 30ºC (Lock and Board, 1994, 1995). There has been an incident of *S.* Typhimurium in eggs in the UK, 120 of 170 people were reported to have a gastrointestinal illness after eating in a large metropolitan building (Mitchell et al., 1989). In one of the Danish incidents, a contamination of toxin-producing staphylococci also was observed in *Salmonella*-contaminated mayonnaise (pH of 6.0) (Meyer and Oxhoj, 1964). In addition, another

pathogen such as *E coli* O157:H7 can survive for 7 days in commercial mayonnaise (Glass et al, 1993).

Chemical food additives such as benzoic acid and sorbic acid are used as preservatives in commercial dressed salad. For human, because these chemical preservatives may affect liver and kidney, the WHO's International Programme on Chemical Safety (IPCS) (1996) suggests a provisional tolerable intake of 5 mg/kg body weight per day. Therefore, natural substances that can eliminate pathogen in non-sterilized food are considered as a new alternative raising more safety on consuming non-sterilized foods.

Table 2.3 Controlled pathogens in mayonnaise and dressings

Significant hazards[a]	*Salmonella* spp. *Escherichia coli O157:H7* *Listeria monocytogenes* *Staphylococcus aureus*
Spoilage	Spoilage can occur due to acetic-acid resistant microorganisms (i.e. certain yeasts and lactic acid bacteria). The major spoilage problems can be controlled by selecting suitable stable formulations, by preventing contamination via raw materials such as egg and the process environment, by hygienic packaging, and chilled storage, and distribution.

[a] In particular circumstances, other hazards may need to be considered.

Source: International Commission on Microbiological Specifications of Foods (ICMSF) (2005)

2.3 Microencapsulation

The advent of advanced technologies leads to new products better qualified for application in pharmaceutical and food industries. Especially in the food field, there are technologies useful for making quality-adding materials for application in food products. Microencapsulation is one of the techniques widely used in various industries in order to make packaging of food ingredients, i.e., flavor, probiotic and vitamin. This technique may transform agents of liquid form to a dry form, which is easy to handle and offers application convenience (Gibbs, 1999; Madene, 2006). Moreover, in term of pharmaceutic a drug microencapsulated technique helps cover a bad smell or a bitter taste, and protect degradation of the active ingredients.

Microencapsulation is a technique of coating specific core substances which can be solid, liquid, or gas, in microcapsules with a polymeric material carrier, i.e., wax, gum arabic, or modified starch, which has to be capable of forming wall structure in order to protect special properties of the core materials during storage. In general, the most physical forms of microcapsules appear in a spherical shape having a diameter from nanometers to millimeters. At present, popular microencapsulation technigues include spray drying, freeze drying, extrusion, co-crystallization, etc.

Studies on microencapsulation started from 1930s. The study leading to the development of microencapsulation procedures for pharmaceuticals was published by Bungenberg de Jong and Kaas in 1931. They prepared gelatin spheres by using coacervation process. During 1930s to 1950s, Green and co-worker also created the gelatin coacervation process, which eventually led to several patents for carbonless carbon paper by using gelatin encapsulated oil phase usually containing a colorless dye precursor (Dziezak, 1988; Shahidi, 1993; Green and Schleicher, 1995).

In the food industries, microencapsulation is a common technique which has been employed for many years. In 1951, Griffin reported about the process for preparation of solid oil concentrate by mixing 20 g of lime oil in 200 g molten sorbitol containing 2% of water. The emulsion of the oil in molten sorbitol was cooled and cut into pellets. The products were similar to the products obtained by co-crystallization

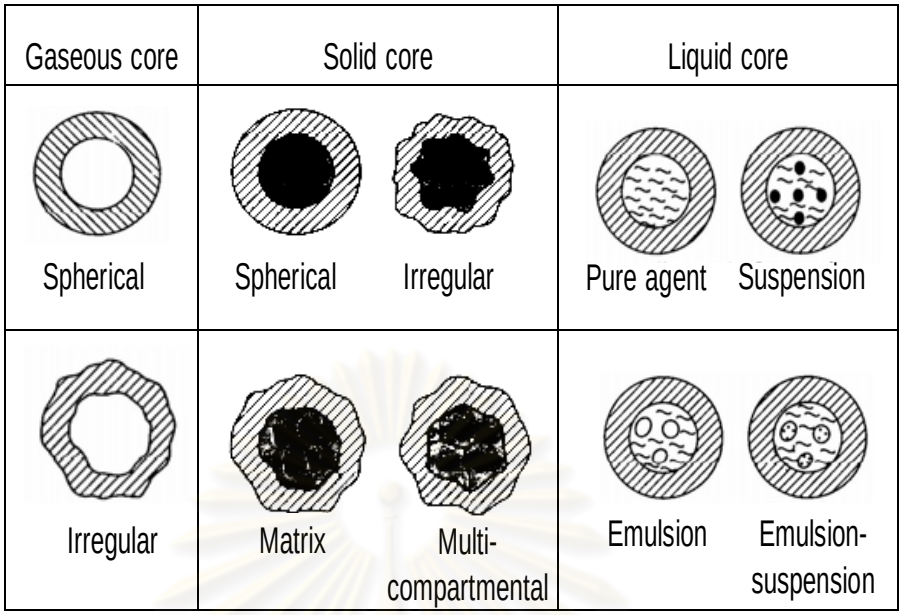
and extrusion (Olsen and Seltzer, 1945). Nowadays, there are several different processes employed for microencapsulaion of food additive, mainly including spray drying, fluidized bed coating, and extrusion. Other processes such as dehydration, coacervation, and cocrystalization are not frequently used (Jafari, 2008).

2.3.1 Microparticles

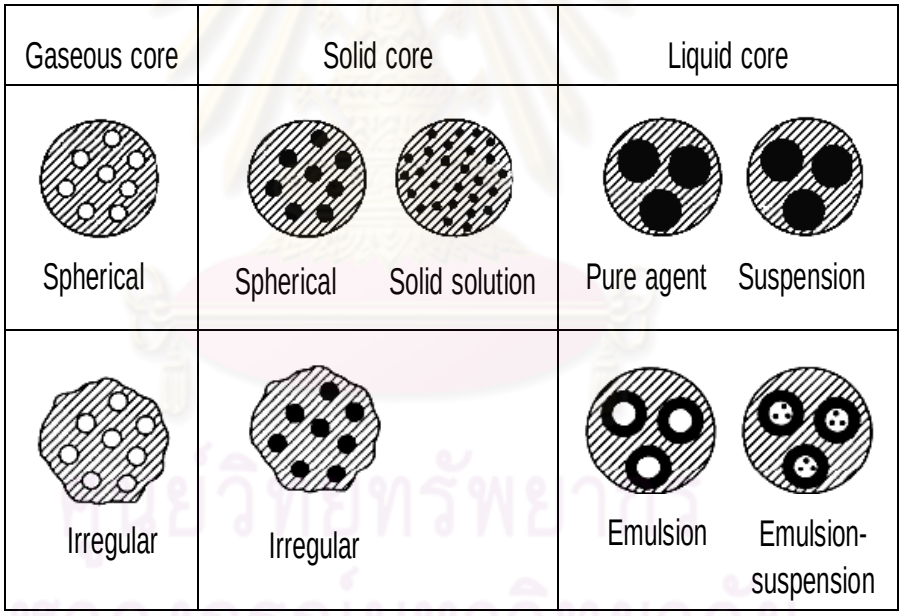
Particles having the diameter between 1 to 5,000 μm are known as microparticles. Microparticles can be divided into two major patterns; microcapsules and microspheres, which may be in either spherical shape or non-spherical shape (Figure 2.3) (Jafari, 2008; Re', 1998).

Generally, microcapsule structure composes of essential ingredients as core materials which are covered by polymeric wall materials. The core materials may contain only one or several ingredients and the wall may be in single layer or multiple layers. Wall surface may be either smooth or rough, sometimes even having holes distributing on particle surface. The cores can be inside the microparticle in form of solid, solution, suspension, or aerosol that is called a reservoir (Arshady, 1992). In case of microspheres, active agents which may be solution, dispersed particle, or molecular agent spread continuously in the wall layers. The wall material can not be clearly separated from the core material (Figure 2.3b) (Mathiowitz et al., 1999).

The study of microencapsulation needs the understanding and knowledge on basic properties of microcapsule, for instant, core and coating material properties, stability, releasability, as well as the microencapsulation process.



(a)



(b)

Figure 2.3 Structures and characteristics of (a) microcapsules (b) microspheres

Source: Mathiowitz et al. (1999)

2.3.1.1 Core materials

Core materials may be in solid, liquid, or gaseous form. In liquid form, they compose of dispersed materials and liquid-soluble agents. In the one hand, the solid cores are compounds with combined active constituents, stabilizer, diluents, excipients, and released rate retardants or accelerator. The liquid core may be non-polar and/or polar substances.

Main core material structures can be classified into two types; single core and multiple cores (Figure 2.4). Single core has high core loading (approximately 90% of total capsule weight), which is prepared by complex coacervation, fluidized bed drying, co-extrusion, and molecular inclusion process. For multiple cores, which are produced principally by spray drying, the core material is dispersed on the wall material and the void is in the particle center (Jafari et al., 2008).

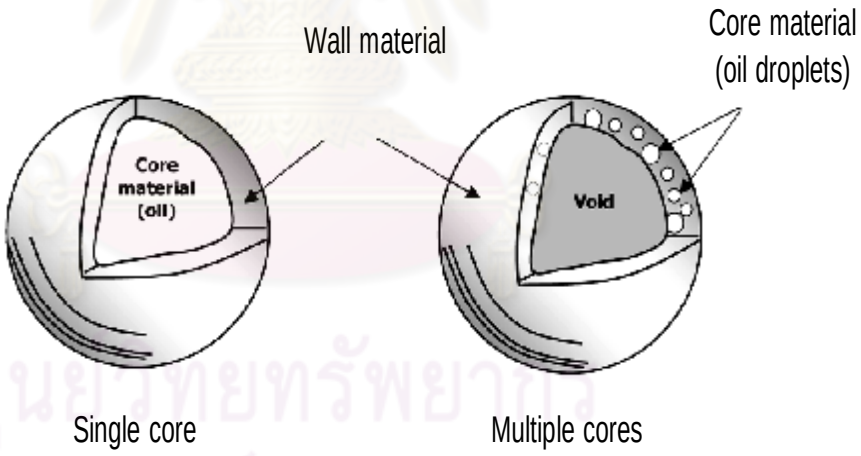


Figure 2.4 Two different types of microcapsule structures.

Source: Jafari et al. (2008)

2.3.1.2 Coating materials

The important criteria for selecting coating materials are as follow;

1. what product patterns are required, such properties as low volatility, good release controlling, or core-protective ability,

2. kind of coating materials that can provide a desire target product, and

3. in case of food ingredients microencapsulation, the materials need to be ‘edible’ and ‘bland’.

Coating materials, which are basically film forming materials, can be chosen form a wide variety of natural or synthetic polymers, depending on material to be coated and required characteristics in the microcapsule product. The composition of coating material can affect the release ability of a particular ingredient. An ideal polymeric material should express the following characteristics;

1. Good rheological properties at high concentration which leads to comfortable working ability.

2. The ability to disperse or emulsify an active material and to stabilize the produced emulsion.

3. Non-reactivity with a wall material used for encapsulation during processing and prolonged storage.

4. Sealing and holding the active ingredient ability during processing or storage.

5. Completely releasing solvent or other materials capability during dissolution.

6. Protection to active materials against environmental condition, i.e., light, oxygen, and humidity.

7. Solubility in solvents that are acceptable in the food industry.

8. Inexpensive food grade status.

Colloids are also common coating materials for food microencapsulation. The selected colloidal materials such as vegetable-based colloids or gum are edible and maintain original flavor of food. Other natural polymeric materials used in food microencapsulation include gelatin, vegetable derived product, milk protein, waxes, and fat. Sometimes, they may be combined with another material to make a new coating material having dominant properties that could satisfy multiple requirements (Table 2.4).

Controlled release methods can be employed in setting free active agents or ingredients at a desired site and at specific rate. These techniques deal with uses of heat, temperature, moisture, pressure, electromagnetic or pH-sensitive additives. The ingredients presenting in microcapsules can be released under an effect of a specific stimulus at a specific stage. For instance, sweeteners susceptible to heat may be released toward the end of baking in order to prevent undesirable caramelization in the baked products. Breaking of coating material may also result from pressure, grind, or other related mechanisms. In addition, the core release depends on diffusion ability or leaching of permanent fluid. Many different releases are related to mechanisms such as diffusion, dissolution (water or solvent), molecular trigger (such as pH), biodegradation, thermal, mechanical, or osmotic stresses. Figure 2.5 shows that osmotic release is triggered by the absorption of water into the microcapsule core. Subsequent swelling ruptures the microcapsule shell (Persyn and Oxley, 2008).

Table 2.4 Different coating materials used in spray-drying microencapsulation of food oils and flavors (Jafari et al., 2008)

Wall materials	Properties	Examples	Encapsulated flavors and oils
Carbohydrates			
- Hydrolyzed starches	Good oxygen barrier, cheap, low viscosity at high solid; no/limited emulsion stabilization	Corn syrup solids, maltodextrins	Citral and linalyl acetate; ethyl caprylate; cheese aroma; orange peel oil
-Modified starches	Very good emulsion stabilization, inexpensive sometimes varying quality, not universally usable owing to regulatory situation	Capsul, N-lok, HI-cap	Meat flavor; fish oil; orange oil; d-limonene; l-menthol; butter oil; cream; black pepper oleoresin; vitamin E
-Gums	Good emulsions, very good retention of volatiles; varying quality, sometimes impurities	gum arabic, mesquite gum	Essential oils; cardamom oil; orange peel oil; linoleic acid; vegetable oil
-Cyclo-dextrins	Very good inclusion of volatiles,excellent oxygen barrier	α-, β-, α Cyclodextrins	Pine flavor; shiitake flavor; d-limonene
Protein			
-Milk proteins	Very good emulsion	Whey protein	Milk fat; soy oil
Other biopolymers			
-Other proteins Soluble soy polysaccharides, chitosan, alginates			Soy proteins, Wheat germ oil; evening primrose oil; fish oil

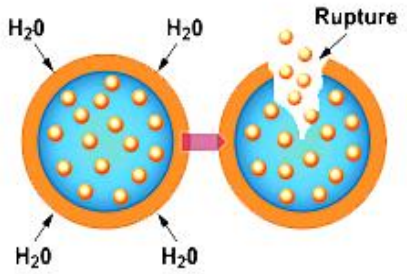


Figure 2.5 Absorption of water into the microcapsule causes swelling rupture of the microcapsule (Persyn and Oxley, 2008).

2.3.1.2.1 Maltodextrin

Maltodextrin is manufactured by partially hydrolyzing starch with acids or enzymes, which breaks the starch into medium-length chains of dextrose (glucose) molecules (Rocha et al., 2005). Maltodextrin may be disguised on labels with different names; sometimes it is referred to as glucose polymers, complex carbohydrate or starch hydrolysis product with different dextrose equivalents (DE) which contains glucose polymer of various lengths. Maltodextrin can be hundreds of sugar molecules in length, much larger than the simple carbohydrate arrangement of glucose. The polymers contained in maltodextrin can be divided into two patterns; linear amylose and branched amylopectin degradation products. Maltodextrin is very different from a typical complex carbohydrate because of its simplified structure of repeating dextrose units. The products in maltodextrin extend from oligomers to macromolecules. Maltodextrins with different DE values exhibit different physicochemical properties such as solubility, freezing temperature, viscosity, etc.

Maltodextrin with a DE value of 10 has been widely used in the food industry as carrier materials in spray drying process because of their several advantages; very good oxygen-barrier, cheap, low viscosity at high solids, ease of drying, aqueous solubility, bland in flavor, light odor and color. Since the molecular weight of DE 10 maltodextrin is about 1800 Daltons with no lipophilic group, their emulsification properties are poor. For this reason, it produces coarse emulsion that result in poor oil retention during drying (Re', 1998).

Beristain and co-workers (2001) studied the efficiency of spray-dried encapsulation by using different blending wall materials; 60% gum arabic (GA), 40% mesquite gum, and a mixture of 40% mesquite gum and 60% maltodextrin. The results showed that the mixture was able to highly encapsulate 83.6% of cardamom essential oil. This concluded that maltodextrin can successfully replace a part of GA as coating material. The combination of maltodextrin and modified starch also reduces the cost of the encapsulating material and enhances the emulsification ability of the encapsulating system (Re', 1998). The degree of protection is directly related to DE of the hydrolyzed starch. Higher DE values produce the powder with excellent encapsulation efficiencies due to its low oxygen permeability (Jafari et al., 2008).

Additionally, other combinations such as proteins and lipids with carbohydrates as encapsulating blend materials have also been investigated. Maltodextrin acted as a matrix film forming material, while the added agent, lipid or protein served as an emulsifying agent (Re', 1998).

2.4 Spray drying

There are several different processes employed for microencapsulation of food ingredients or food additives. The main processes used nowadays include spray drying, fluidized bed coating, extrusion, and coacervation. Since late 1950s, spray drying has been the most common technique in food industries owing to their many advantages, for example, process economics, flexibility, instrument availability, and the ability to yield good quality products. The microencapsulation by spray drying deals with the dispersion of active agents in carrier material solution in order to be sprayed into a hot chamber. Spraying by means of rotary atomization or pressure injection results in lipid droplets with high surface to volume ratio that leads to rapid evaporation (Re', 1998). The mixture is then converted into powder or dry granule due to evaporation of water leaving the polymeric materials encapsulating the active ingredient.

2.4.1 The spray drying operation

Dehydrating fluid by spray drying is a common engineering practice. In general, a spray drying is divided into main four stages, (i) atomization of feed into a spray dryer, (ii) sprayed liquid-air contact, (iii) dehydration (evaporation of water), and (iv) separation of dry product (Figure 2.6).

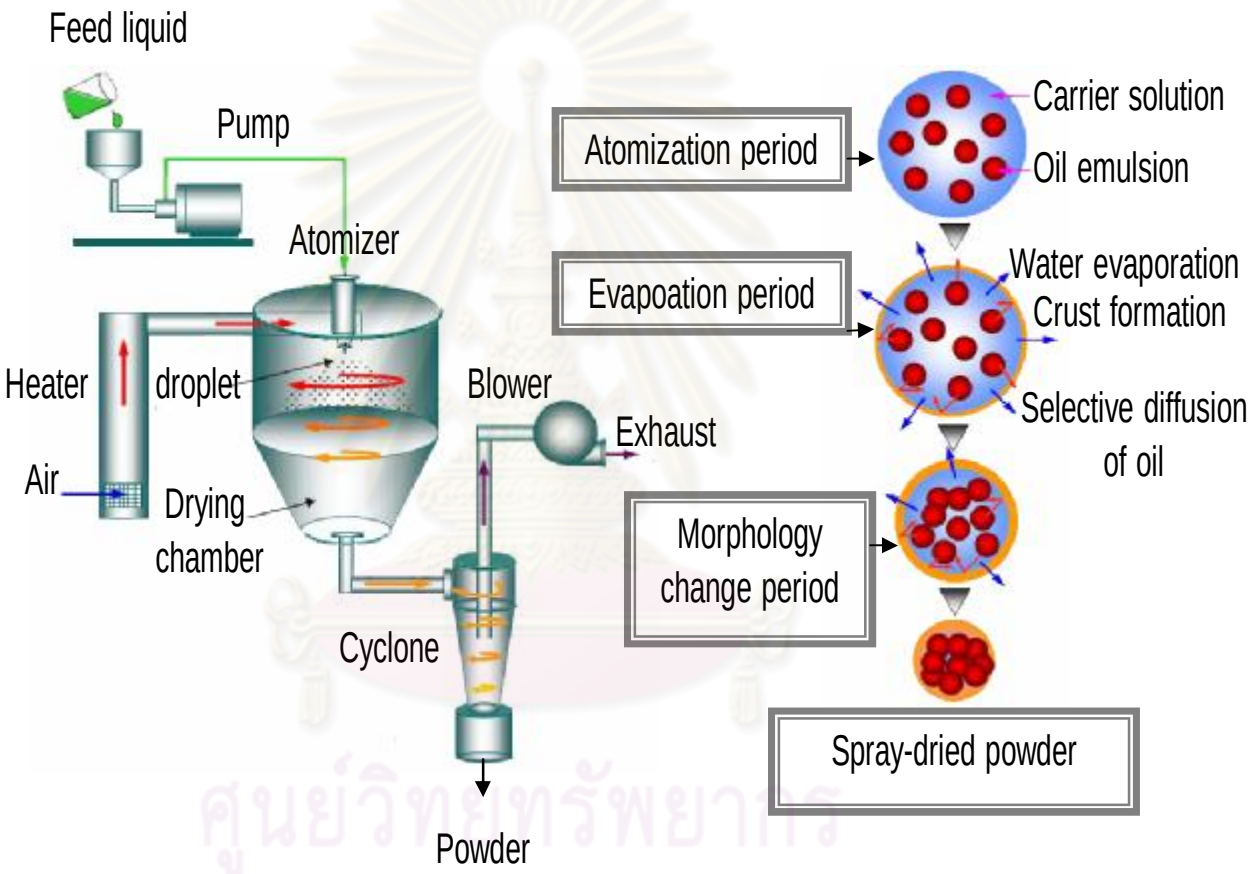


Figure 2.6 Schematic presentation of cocurrent spray drying equipment

Source: Modified from Soottitantawat (2005)

The spray drying begins with the atomization of a liquid feedstock into a spray of droplets. Next, droplets are put in contact with hot air in a chamber. Evaporation of moisture from droplets and formation of dry microcapsules proceed under controlled temperatures and airflow conditions, which can be described about in more details of stage as follows;

-Atomization

The aim of this stage is to create effective heat-transferring at contact surface between hot air and liquid feed by optimizing heat and mass transfer. Available atomizers can be classified according to the nozzle design mainly used in industrial drying, which include rotary (atomization by centrifugal energy), pressure nozzle (atomization by pressure energy), and two-fluid nozzle (atomization by kinetic energy). The selection criteria of atomizer depend upon the natural properties of feed such as viscosity, and desired characteristic of dried product (Gharsallaoui et al., 2007). Dried-particle size correlates with several factors which are feed rate, viscosity, and surface tension of initial liquid. Increasing feed rate causes an increase in particle size at a fixed-energy amount. Other factors such as types of atomizer and rotational speed of atomizer also have an impact on particle size.

-Hot air contact and evaporation of droplet water

This stage involves dehydration of the water in the liquid feed to generate dry powder. When the liquid is sprayed in the chamber in the same direction as the flow of hot air (typically 150 to 220^{o}C for the inlet air temperature), evaporation immediately occurs after droplet-hot air contact so as to convert liquid droplet into solid form at moderate temperatures (50 to 90^{o}C) (Gharsallaoui et al., 2007). In case of thermo-sensitive products, the drying process can be applied as the counter-current drying in which hot air flows in the opposite direction of the sprayed liquid. Heat transfer between air and droplet results from balances of temperature and vapor partial pressure. Basic theory of drying composes of three main steps which may be different in term of duration depending on the feed nature or other related factors such as inlet temperature and feed rate. After liquid-air contact, the droplet temperature is increased to

a constant value by heat transfer process. The droplet water is then dehydrated at a constant temperature and a water vapor partial pressure. Finally, when the temperature reaches a critical value, a dry crust is formed at the droplet surface, drying is then theoretically ended as soon as the particle temperature turns equal to that of the air (Gharsallaoui et al., 2007).

-Dry product separation

After evaporation stage, the final product is separated by means of a cyclone placed outside the drying chamber. The most dense microcapsules are collected at the base of the drying chamber. The finest particles pass through the cyclone in order to be separated from the humid air with both filters; bag houses and chemical scrubbers that are used to remove the finest powder and the remaining powder (or any volatile particles e.g. flavoring) respectively (Gharsallaoui et al., 2007).

2.4.2 The spray-dried particle properties

In general, the dried particles are spherical with a narrow size distribution and with holes distributing on the surface. The technological parameters such as concentration of polymeric solution to be sprayed, inlet and outlet air temperature, feed rate, air flow rate, heating, and exhausting have an impact on the acquired product properties that include particle shape, size distribution, bulk density, particle density, porosity, moisture content, flowability, stability, dispersability, friability, product yield, activity retention, aroma and flavor (Newton, 1966). These properties can possibly be altered and controlled by modifying the parameters involved in the spray drying process.

Conte et al. (1994) studied the effect of the inlet and outlet temperatures, feed rate and of starting polymeric solution concentration on the characteristics of diazepam loaded poly-D,L-lactide microparticles. The particles are evaluated for production yield shape, size and release behavior. The best tested conditions are the highest feed rate and temperature. Lower moisture contents were observed at higher drying

temperatures. Other physical and chemical properties such as total retention and releaseability also depended on the spray drying parameters and composition of feed.

Other essential parameters that are found to affect the product properties significantly are nozzle size and inlet air temperature. Wan et al. (1990) produced coated theophylline microcapsules using hydroxypropylmethylcellulose (HPMC) as the coating polymer by a spray drying process. Their results showed that an increase in nozzle size or a decrease in the air to liquid diameter flow ratio improved some of the flow properties. With an increase in inlet air temperature, there was a corresponding improvement in the flow properties and a reduction in dissolution rate. The dissolution profile indicates an effectiveness of coating, that is, a slower dissolution rate indicates a better coating. In addition, the type of feed used was also important. The suspension feed showed better release behavior and flow properties than the solution feed (Wan et al., 1990).

Sometimes, product properties result from the co-functions of various processes. For instance, Broadhead et al. (1994) evaluated the joint effects of various processing and formation on the properties of spray dried beta-galactosidase, the residual enzymatic activity and the product yield were significantly affected by the processing variables. Their results showed that the product yield directly varied with the drier outlet temperature.

Each parameter may have a different impact on the particle size. The particle size could be reduced with an increase in the energy available for atomization, i.e., rotary speed, nozzle pressure, or air to liquid flow ratio in a pneumatic atomizer. An increase in feed rate causes a raise in the microcapsule size. The effect of temperature on the size associates with the kind of coating material that has different structures. For instance, an increase in drying air temperature caused the mean diameter of particles coated by film forming to be reduced to a greater extent compared with those coated by crystalline material. Newton (1996) reported that the particle size of some materials increased with increasing drying air temperature. Moreover, high drying air temperature also associated with lower bulk densities (Master, 1979).

The advantages of spray drying

The main advantages of spray drying are presented as following;

1. Spray drying is a continuous process and a single step operation from liquid feed to drying product.

2. The process is adaptable to fully automatic control.

3. Specific dried particle characteristics are obtained by designing and operating dryer to get the required product form and properties.

4. Both heat susceptible and heat resistance material can be applied in this process

5. Feedstocks can be of various characteristics such as solution, slurry and thixotropic paste.

6. In spray dryer designs, corrosion is prevented because the material does not contact the instrument surface until it is dry. The extremely rapid evaporation cools the inlet gas near its outlet temperature, and there are few moving parts. From these reasons, the maintenance cost is reduced.

7. Labor cost is low since only one operator is required.

2.4.3 The Initial emulsion

Initial emulsion is one of the key steps in spray drying encapsulation of oil and flavors. This emulsion plays a major role in determining the retention of volatiles and surface oil content of the final encapsulated powder. The related parameters to consider are total solid concentrations, viscosity, stability, droplet size, and emulsification method.

Emulsion stability has an impact on the encapsulation efficiency of oil and flavors. The better emulsion stability indicates higher encapsulation efficiency. Liu et al. (2001) studied the effect of emulsion stability on the retention of emulsified hydrophobic flavor during drying. They found that some factors such as the initial carrier solid concentration and drying air temperature influenced the retention. The retention increased with the increase in the carrier solid concentration. When air temperature was high, a crust formed at the droplet surface so quickly that the flavor could not evaporate easily.

The emulsion droplet size has a considerable effect on the encapsulation efficiency of oil during encapsulation of oils and flavors during spray drying. The encapsulation efficiency of the core material can be improved with decreasing the droplet size to lower than 1.0 μm. The major advantage of producing a finer emulsion is higher stability, which is critical during spray drying. Besides, the emulsion size may affect the characteristics of the final microcapsules such as surface oil and total oil content as well. Risch and Reineccius (1995) reported that a smaller emulsion droplet size yielded a higher retention and lower surface oil on the dried powder of gum arabic or modified starch and orange oil emulsions, hence powders with longer shelf life. Some work (Minemoto et al., 2002) also showed that the encapsulated linoleic acid with smaller emulsion size oxidized more slowly than powders with larger droplet emulsion size, because of the lower surface oil content of the dried particles. This is similar to the results obtained by Soottitantawat et al. (2005) who revealed that increasing diameter of orange oil emulsion resulted in a decrease in the retention of orange oil and its active

composition, d-limonene, for different coating materials (e.g., gum arabic, Hi-Cap, and maltodextrin) (Fig 2.7).

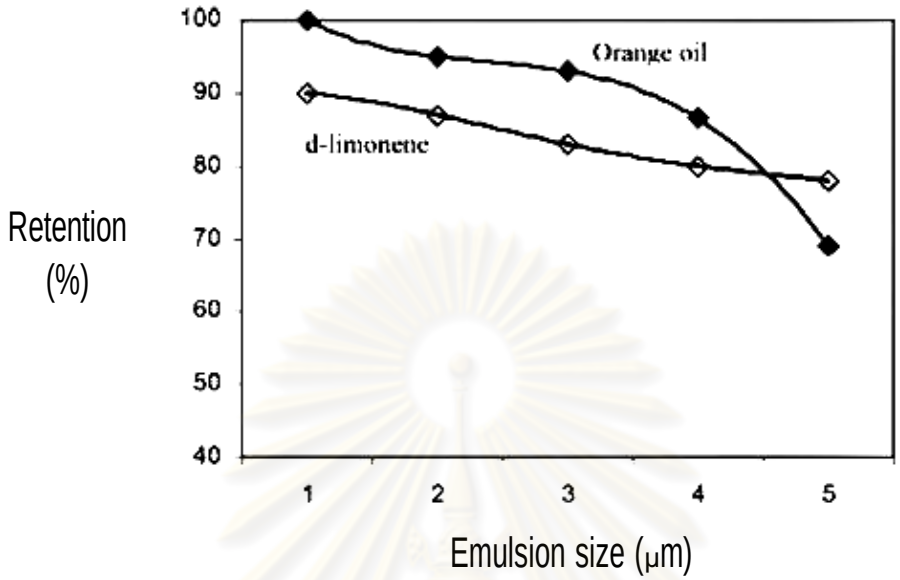


Figure 2.7 Influence of emulsion droplet size on the retention of flavors during spray drying encapsulation of orange oil and d-limonene.

Source: Jafari et al. (2008)

2.4.3.1 Polyoxyethylene surfactants (Polysorbate)

In general practice, the surfactants are employed in macroemulsion formation. Polyoxyethylene surfactants or polysorbate, commercially known as Tween®, are one of the emulsifiers commonly used in some pharmaceutical and food products. Polysorbates are a nonionic surfactant and oily liquids derived from polyethylene glycol (PEG)-ylated sorbitan (a derivative of sorbitol), which are esterified with fatty acids and are often called Tween. There are different polysorbate series commonly used, i.e.,

-Polysorbate 20 (Tween®20 or polyoxyethylene (20) sorbitan monolaurate)

-Polysorbate 40 (Tween®40 or polyoxyethylene (20) sorbitan monopalmitate)

-Polysorbate 60 (Tween®60 or polyoxyethylene (20) sorbitan monostearate)

-Polysorbate 80 (Tween®80 or polyoxyethylene (20) sorbitan monooleate)

The number 20 following the *polyoxyethylene* part refers to the total number of oxyethylene -(CH_2CH_2O)- groups found in the molecule. The number following the *polysorbate* part is related to the type of fatty acid associated with the polyoxyethylene sorbitan part of the molecule. Monolaurate is indicated by 20, monopalmitate is indicated by 40, monostearate by 60 and monooleate by 80.

Polysorbate 20 and 80 belong to a class of food additives. A summary of the major physical properties of them is shown in Table 2.5. They are approved for use in specific products and are generally recognized as safe (GRAS). They are well tolerated upon oral administration and are practically non-irritating, possessing very low toxicity potential. As a food additive, Tween®80 is used in ice creams, pickles, vitamins/mineral preparations, whipped toppings, gelatin desserts, cottage cheese, barbecue sauce, etc. Generally, the acceptable daily intake (ADI) of both Tween®80 and Tween®20 set by world health organization (WHO) is 25 mg per kilogram of body weight.

Table 2.5 Major physical properties of the Tween®20 and Tween®80

Properties	Tween®20	Tween®80
Chemical name	Polyoxyethylene (20) sorbitan monolaurate	Polyoxyethylene (20) sorbitan monooleate
Molecular weight	1228 g/mol	1310 g/mol
Density (g/ml)	1.105	1.064
Critical micelle concentration (mM)	0.059 (0.0072%)	0.012 (0.0016%)
Hydrophilic Lipophilic Balance (HLB)	16.7	15.0
Cloud point (°C)	76	65
Viscosity at 25 °C (cP)	330	425
Phase	Clear liquid	Golden-yellow viscous liquid

Source: Malgorzata et al. (2007)

2.5 Essential Oil Microencapsulation by Spray Drying

There have been many works (Brenner et al., 2000; Beristain et al., 2001) in the literature dealing with general issues of essential oil microencapsulation by spray drying. Essential oils retain volatile oil component which are a class of volatile organic compounds derived from plants through distillation. The oils are called "volatile" because they can evaporate (become airborne) at low temperatures. Therefore, essential oil microencapsulation by spray drying is employed to preserve their biological activity and their load by containing oil inside a carrier or a wall material. Several studies (Re', 1998; Beristain et al., 2001) reported the preparation of various essential oil microencapsulation with different coating materials. Beristain et al. (2001) prepared spray-dried microcapsules of cardamom essentail oil with mesquite gum in order to investigate the ability of mesquite to act as an encapsulating agent for the production. They reported that the stability against drop coalescence of emulsions was elevated when increasing oil or decreasing gum for all the gum to oil ratios studied (1:5, 1:4, and 1:3). High oil retention (83.6%) was attained when proportion of 1:4 oil:gum was used. This confirmed that the microcapsules had a good flavor encapsulation ability and could be readily used as a food ingredient.

In another study, peppermint essential oil microcapsules were prepared by spray-drying emulsion of oil in modified starches (Baranauskiene et al, 2007). Various modified food starch matrices were used as an encapsulating agent, for example chemically *n*-octenyl succinic anhydride (OSAN)-modified starches HI-CAP 100, N-LOK (starch with corn syrup added), and CAPSULE (derived from waxy maize) and the acid- and/or enzyme hydrolyzed starches (dextrins), ENCAPSULE 855 (refined from tapioca and mized starch), CRYSTAL TEX 627 (refined from tapioca starch), CIEmCap 12633 (stabilized and acid–thinned instant waxy maize starch), CIEmCap 12634 (spray-dried waxy maize starch ester), CIEmCap 12635 (stabilized and acid–thinned instant waxy maize starch). The results revealed that all *n*-octenyl succinic anhydride (OSAN)-modified starches had higher emulsification and encapsulation efficiencies of peppermint essential oil than hydrolyze starches (dextrins). Loss of the essential oil (EO) volatiles during storage was more intense at a higher water activity (a_w) level. The

effect of a_w on the release of EO was related with the structural changes of the coating matrices. The lower mobility of EO molecules was found in the glassy state of the capsule matrices. At high a_w levels, the matrix started to plasticize, resulted in an increase of release rate of the possibly higher mobile EO.

Jafari et al. (2008) reported that the loss of some volatiles during spray drying encapsulation is inevitable. Other related parameters, for example, the properties of wall and core materials (molecular weight, vapor pressure and structure) and the prepared emulsion along with the drying process conditions have an impact on the efficiency and retention of the core compounds. The increase in molecular size generally results in slower diffusion rate. Additionally, the retention of volatiles also depends on their polarity. The more polar compound exhibits less retention which could be explained by the greater solubility of polar compounds in water.

The retention of volatiles depended on their molecular weight, relative volatility, polarity and type. Goubet et al. (1998) stated that the retention of aroma compound with various functional groups is in the order of acids< aldehydes< esters≤ ketones≤ alcohols with acids having the minimum retention. The other possible parameter such as interactions between the volatiles and wall materials might associate with physical or physicochemical interactions including insoluble complex association and molecular association of the coating material with the volatile through hydrogen bonding, which could influence the formation of the interfacial film at the interface of oil-in-water stabilizing the emulsion and may affect the retention indirectly.

In many of the published works (Reineccius, 1988; Sankarikutty et al., 1988; Risch, 1995), the optimal core to coating material ratio of 1:4 (20% core at the final encapsulated powder) for various materials like gum arabic and modified starches was reported. In contrast, Brenner et al. (2000) showed in their patent that the core to coating material ratio of 75% flavors could be produced by using sorbitol as a plasticizing wall material but there has been no commercial products using this patent.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and instruments

3.1.1 Materials

1. Garlic oil (Thai-China Flavours and Fragrances Industry Co., Ltd., Thailand)
2. Tween®20 and Tween®80 (Merck, Germany)
3. Maltodextrin DE10 (Berli Jucker Public Co., Ltd., Thailand)

3.1.2 Test microorganisms

1. *Escherichia coli* ATCC 25922 (TISTR culture collection, Thailand)
2. *Salmonella* Typhimurium ATCC 13311 (TISTR culture collection, Thailand)
3. *Staphylococcus aureus* ATCC 25923 (TISTR culture collection, Thailand)

3.1.3 Chemicals

1. Ethyl alcohol	Merck, Germany	A.R. grade
2. Nutrient agar	Himedia, India	A.R. grade
3. Nutrient broth	Himedia, India	A.R. grade
4. Glycerol	Ajax Finechem, Australia	A.R. grade
5. Petroleum ether	Labscan Asia Co. Ltd., Thailand	A.R. grade
6. Plate count agar	Britania, Argentina	A.R. grade

7. Peptone	Merck, USA	A.R. grade

3.1.4 Instruments

1. Weighing scale (Sartorius A200S, Mettler-Toledo, Switzerland)
2. Hot air oven (Model 600, Memmert, Gmiott Co. KG, Germany)
3. Magnetic stirrer (Framo®, Germany)
4. Rotary evaporator (N-N Series, Rikakikai Co. Ltd., Japan)
5. Goniometer (FTA200 series, First Ten Angstroms, USA).
6. Spectrophotometer (Genesys 20 Model 4001/4, Thermospectronic, Rochester, New York, USA)
7. Hand-held homogenizer (model x10/25, Ystral, Germany)
8. Laminar flow 'clean' (Model V6, Lab service Ltd., Thailand)
9. Water activity analyzer (AquaLab Series 3, Decagon Devices, Inc., USA)
10. Refrigerator (Model Compakt 880(B)H, Foster Refrigerator Ltd., U.K.)
11. Vortex mixer (Model G-560E, Scientific Industries, Inc., Bohemia N.Y., 11716, USA)
12. Thermostatic Water bath (Model WB 14, Memmert, Schwabach, Germany)
13. Autoclave (Model Autoclave ES-315, Tomy Seiko Co., Ltd., Tokyo, Japan)
14. 4-digit precision weighing balance (Model AG 204, Melter Toledo, Switzerland)

15. Microwave (Model 000502174, Thai Cityelectric Co., Ltd., Thailand)

16. pH meter (Model Cyberscan pH 1100 Bench, RUTECH instruments pte. Ltd, Singapore)

17. Blender (HR 1791, Phillips, Indonesia)

18. Scaning electron microscope (model JSM-5410LV, JEOL, Japan)

19. Particle size analyzer (Master sizer 2000 version 5.22, Malvern Instrument Ltd., U.K.)

20. Viscometer (Viscometer rheology international model RI:2:L, Shannon Ltd., Ireland)

21. Colorimeter (Minolta Chroma Meter model CR-400, Osaka, japan)

22. Infrared moisture analyzer (Mettler Toledo (MJ33)

23. Spray dryer (GEA Niro Inc., USA)

3.2 Methods

3.2.1 Determination of critical micelle concentration (CMC) of Tween® in 20 g/dL maltodextrin

3.2.1.1 Preparation of Tween® in maltodextrin solutions

Tween®20 and Tween®80 was dissolved in maltodextrin solutions at different concentrations (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.4, 0.6, 1% w/v). The solutions were transferred into test tubes, which were then determined for its surface tension by a goniometer.

3.2.1.2 Determination of CMC

The surface tension of all Tween®in maltodextrin solutions (at every concentration) was determined by using an automated goniometer equipped with the FTA 32 V 2.0 software. The procedure is shown in appendix A1. The measurements were done in duplicate. CMC is the point at which the surface tension of the solution changes sharply. The experiment was conducted in 2 replications.

The steps in determination of critical micelle concentration of Tween® in 20 g/dL maltodextrin are shown in Figure 3.1.

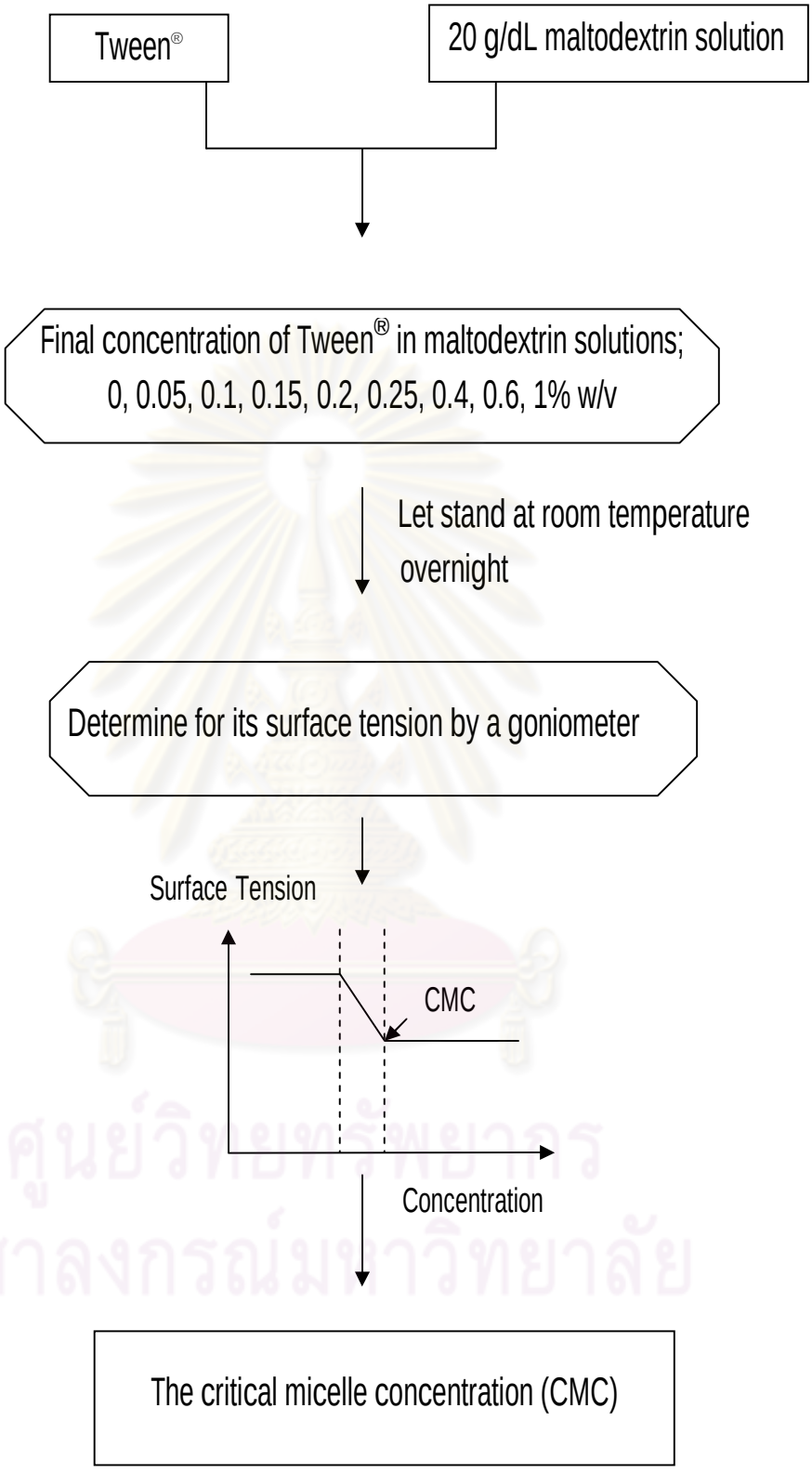


Figure 3.1 Steps in the determination of critical micelle concentration of Tween® in 20 g/dL maltodextrin.

3.2.2 Determination of stability of garlic oil emulsions

3.2.2.1 Preparation of garlic oil emulsion

The emulsions were prepared by mixing Tween®20 in 20 g/dL maltodextrin solutions to obtain the final Tween® concentration of 0.6%, 0.8%, and 1% w/w. Garlic oil was then added to the solutions to get oil to maltodextrin ratios of 0.1:1, 0.15:1, and 0.2:1 w/w. The mixtures were then homogenized using a hand held homogenizer at different rotational speeds (13,000, 19,000, and 24,000 rpm for 5 and 10 min).

3.2.2.2 Emulsion stability analysis

Twelve (12) mL of each emulsion was placed into capped vials and stored at room temperature (25°C) for 48 hours. The separating oil layer of emulsion was recorded at 48 hours. The measurement was performed in 3 replicates.

3.2.2.3 Oil droplet size analysis

Size distribution of oil droplets dispersed in emulsion was carried out for each sample that was stored at 4°C by using a particle size analyzer. Each sample was added to 700 mL of water until value reached an obscruration limits. The surface-average diameter, D[3,2], was determined following equation 3.1 (Sherman, 1968; Beristain et al., 2001):

$$D[3,2]=(\Sigma n_i d_i^3 / \Sigma n_i d_i^2) \quad (3.1)$$

Where, n_i is the number of droplets with diameter d_i.

The measurement was done in triplication.

The steps in determination of stability of garlic oil emulsions are shown in Figure 3.2.

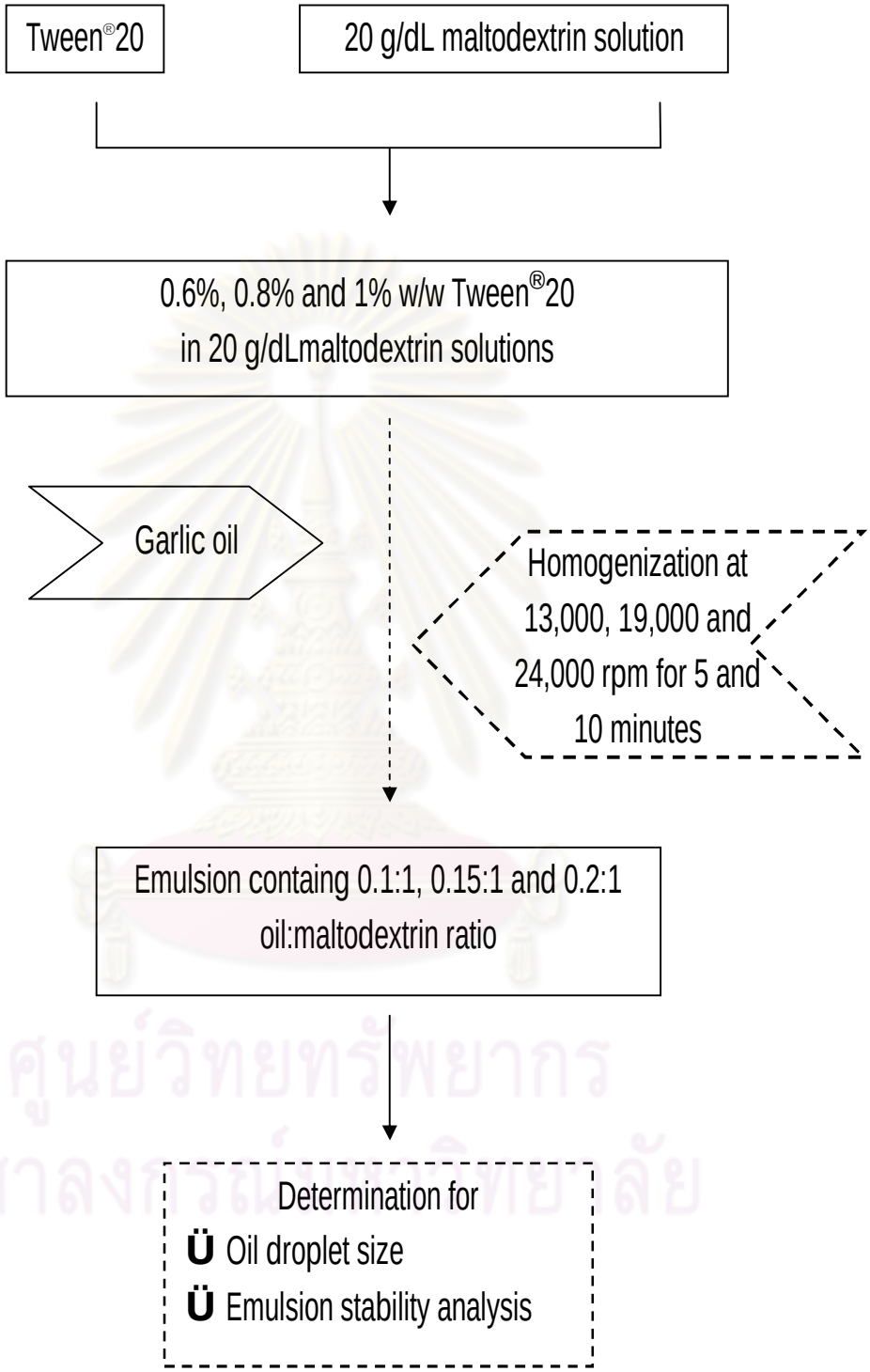


Figure 3.2 Steps in the determination of stability of garlic oil emulsions.

3.2.3 Spray drying of garlic oil emulsions

One thousand (1000) mL of garlic oil emulsion was prepared by using a hand held homogenizer. The emulsion was spray dried at the operating conditions (Table 3.1) which were obtained according to the method that was modified from that of Beristain et al. (2001). The dried microcapsules were packaged in an aluminum foil laminated pouch (OPP/AL/PE/LLD 100 μm) to prevent degradation of the formulation by moisture during storage. All measurements were carried out in 2 replicates. The steps in spray drying of garlic oil emulsions are shown in Figure 3.3.

Table 3.1 The operating conditions of spray drying

Parameters	Value
Inlet temperature (^{o}C)	120, 160, 180 and 200
Feed rate (mL$\cdot$ min^{-1})	25$\pm$5
Pressure (bars)	3

3.2.4 Evaluations of physical and chemical properties of spray dried powders

3.2.4.1 Powder morphology and particle size analysis

A small amount of microcapsules were placed on one surface of a double-faced adhesive tape that sticks to a stub which was used as the sample support. The stub sticked microcapsules were coated with gold under vacuum condition and then observed using a scanning electron microscope (SEM) at 1000x, 1500x and 3500x magnifications in order to determine the particle size and to observe particle morphology. Average size of at least 50 particles was determined for each sample using a SemAfore program (Version 4.01 demo, JEOL, Finland). The procedure is shown in appendix A2. The measurements were done in triplicate.

3.2.4.2 Bulk density determination

Bulk density of microcapsules was investigated by measuring the volume of a certain mass of microcapsules sample that had been passed though a screen into a cylinder. The procedure was modified from the tapping method that was described by Beristain *et al*. (2001) and shown in Appendix A3. The bulk density of dried particles was calculated from the ratio of mass (g) and volume (mL). The measurements were done in replication.

3.2.4.3 Moisture content determination

Moisture content of dried microcapsules was determined following method number 925.10 of AOAC (1995) as described in appendix A4. The measurements were performed in triplication.

3.2.4.4 Water activity determination

The water activity (a_w) of microencapsules was determined by using a water activity analyzer following the step in Appendix A5. The measurements were performed in triplication for each sample.

3.2.4.5 Solubility test

The solubility of all dried-microcapsule samples were measured following the method that was modified from the procedure described by Jangchud and Chinnan (1999) (Appendix A6). All measurements were performed in duplication.

3.2.4.6 Total oil content determination

Total oil content was observed according to the procedure that was modified from that of Hogan et al. (2001). The dried sample (approximate 2 grams) was added with 250 mL petroleum ether. The sample was shaked by a shaker at 200 rpm for 6 hours. The sample was then filtered through Whatman no.41 filter paper into a round bottom flask and the solvent was distilled off from the flask by using a rotary evaporator. The solvent was evaporated completely in a hot air oven at 50˚C for

2 hours. After that the flask was cooled down in a desiccator for an hour and reweighed. Total oil in microcapsules was calculated following equation 3.2.

Total oil (%) = (weight of fat (g) / (weight of sample-moisture) (g)) x 100 (3.2)

3.2.4.7 Bioactive compound analysis

Approximately 1 g of microencapsules and garlic oil were extracted with 5 mL of ether twice. The ether extracts were combined and were then evaporated by using a rotary evaporator until transparent oil was obtained. The oil yield percentage was calculated and the bioactive compounds having antimicrobial activity, which are diallyl disulfides (DADS), diallyl trisulfides (DATS) and cyclic sulfurs (S_8) were analyzed by gas chromatography technique (GC) which was operated under the conditions shown in Table 3.2

Table 3.2 Operating conditions of gas chromatography technique (GC)

Column: CP-sil 5	
Maximum temperature: 300 ^{o}C	
Equivalent column: DB-1, HP-1	
Detector: FID	
Carrier gas: N_2	
Initail temperature 50^{o}C, 2 min	Temperature increasing rate#1, 5^{o}C/min
2nd temperature 110^{o}C, 0 min	Temperature increasing rate#2, 0.5^{o}C/min
3rd temperature 111^{o}C, 1 min	Temperature increasing rate#3, 3^{o}C/min
4th temperature 150^{o}C, 0 min	Temperature increasing rate#4, 1^{o}C/min
5th temperature 165^{o}C, 0 min	Temperature increasing rate#5, 10^{o}C/min
Final temperature 280^{o}C, 0 min	

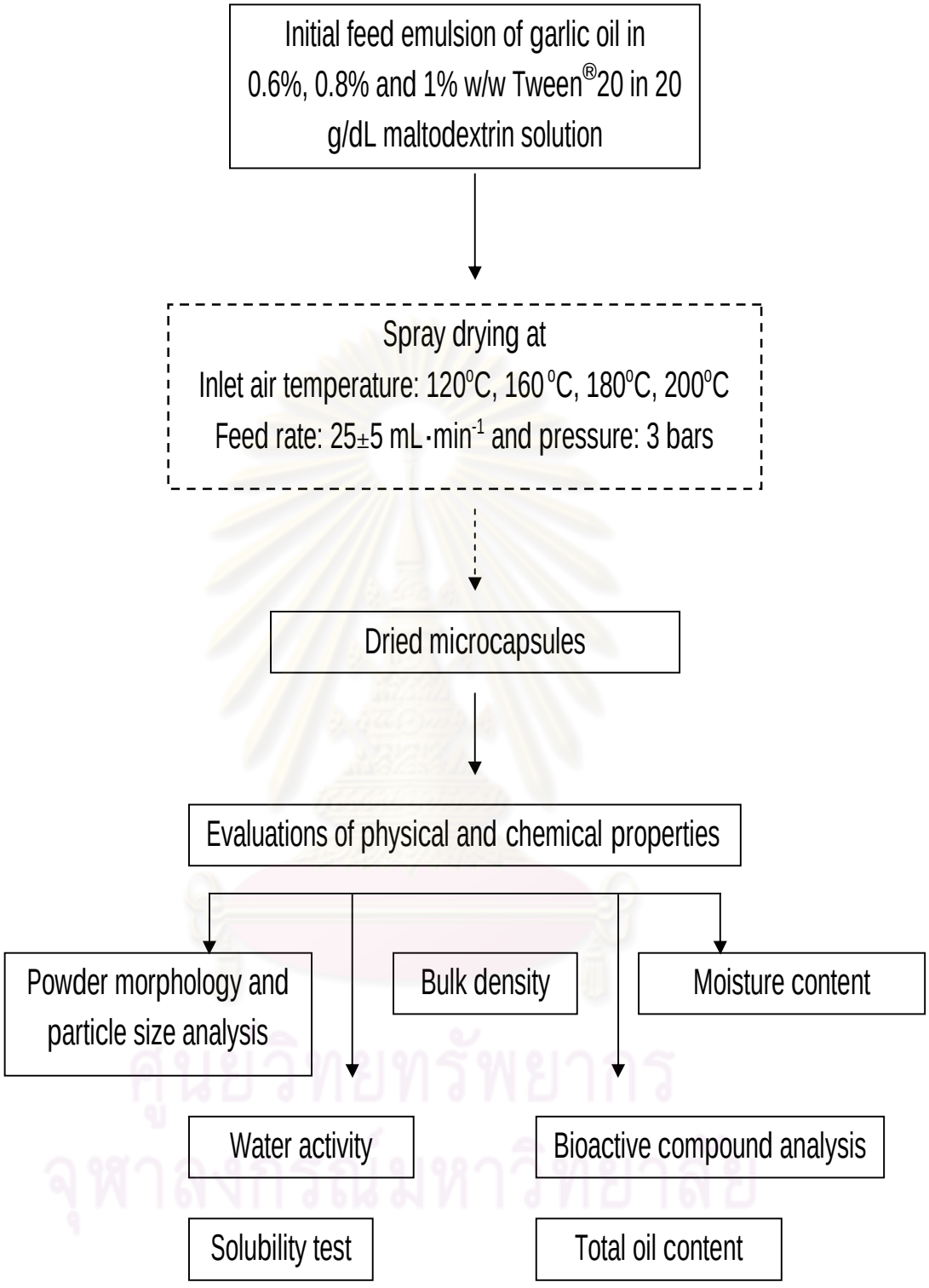


Figure 3.3 Steps in the determination of physical and chemical properties of garlic oil microcapsules.

3.2.5 Antimicrobial assay

3.2.5.1 Test microorganisms preparation

Escherichia coli ATCC 25922, *Salmonella* Typhimurium ATCC 13311 and *Staphylococcus aureus* ATCC 25923 was cultivated in nutrient broth (NB) at 37˚C until bacteria grew up to mid-log phase (10^7-10^8 CFU/mL). The cell concentration density was measured by a spectrophotometer at 600 nm. The target absorbance that indicated mid-log phase growth was 0.4. Bacterial cultures at the mid log phase were diluted to 10^6 CFU/ mL for subsequent use in the experiment.

3.2.5.2 Minimum Inhibitory Concentration (MIC) of emulsion

MIC of garlic oil emulsion was determined by using agar well diffusion method described by Parente et al. (1995) that is exhibited in Appendix B1. Five (5) mL of emulsions containing Tween®20 at different concentrations (0.6%, 0.8%, and 1%), were prepared and transferred into 5 sterile test tubes that contained 5 mL of distilled water to make two-fold dilution series. Sample of 0.25 mL from each tube was taken with a micropipette and transferred into agar wells in a plate containing semi-solid agar (1%) and each indicated microorganisms (*Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 13311, and *Staphylococcus aureus* ATCC 25923). The sensitivity to garlic oil emulsion was classified by the diameter of the inhibition halos as sensitive for diameters at 0.2 mm or more than 0.2 mm that did not include diameter of cock border (0.8 mm) (Ponce et al., 2003)

3.2.5.3 Minimum Inhibitory Concentration (MIC) of microcapsules

Garlic oil were prepared and transferred into 5 sterile test tubes, which contained 5 mL of distilled water. Each microencapsulated samples were added to the tubes to make two-fold dilution series. Then, all tubes were mixed by a vortex mixer and placed at room temperature (~25^{o}C) for an hour. The details of experimental procedures for the agar diffusion assay were similar to that shown in 3.2.5.2.

3.2.6 Effect of temperature on microbial growth inhibition ability of garlic oil

One (1) mL emulsion was added to sterile microtubes. Each microtube was immersed in a temperature controlled water bath at 70, 80 and 90°C for 0.5 to 25 min. After the specified time, the tubes containing sample were cooled down in an ice-cold water bath. The antimicrobial activity of the heated garlic oil was determined by the agar diffusion method described in 3.2.5.2.

3.2.7 Evaluation of oil release in water from microcapsules

The procedure for the release test was modified from Dawson et al. (2003). Five (5) g of garlic oil microcapsules was dissolved in 50 mL of sterile distilled water to obtain the final oil to aqueous phase concentration of 3.6 g/mL. Two hundred and fifty (250) μL of sample was then taken with a micropipette after 5, 10, 15, 10, 15, 20, 25, 30, 40, 50 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24 and 48 hours. The concentration of garlic oil which was released into water was revealed as the width of inhibition zone (cm) by the agar diffusion method described in 3.2.5.2. The experiment was done in 4 replications.

3.2.8 Application of garlic oil microcapsules in salad dressing

3.2.8.1 Preparation of salad dressing

Salad dressing was prepared following a standard recipe shown in Appendix C1. The dressing composed of 39.62% w/w water and 23.53 %w/w oil. The aqueous phase contains vinegar, egg yolk, lime, condensed milk, and milk (Table 3.3). 3.962 g of microcapsules was added to the dressing to make the final concentration of 3.962×10^{-2} g/g.

Table 3.3 Volume of aqueous phase in salad dressing model on 1 unit of recipe

Composition	Aqueous phase(g)	Aqueous phase (%)
Vinegar	9.55	0.90
Egg yolk	85	8.00
Lime	25	2.35
Condensed milk	104.9	9.87
Milk	196.56	18.50
Total salad dressing (1,062.5 g)	421.01	39.62

3.2.8.2 Color determination

Color of salad dressing were measured by a colorimeter in the CIE LAB color system (L*, a*, and b*), using D65 as the light source. L* is the lightness coordinate, a* is the red/green coordinate, with +a* indicating red, and -a* indicating green, and b* the yellow/blue coordinate, with +b* indicating yellow, and -b* indicating blue. Six measurements at six different positions were done for each sample.

3.2.8.3 Sensory assessment of dressing containing garlic oil microcapsules

Samples of fifty (50) mL of salad dressing containing garlic oil microcapsules were stored at room temperature (~25˚C) for up to 7 days. The samples were coded with three digit random number before they were presented to 50 assessors. The assessors were asked to evaluate the samples for their color, odor, texture and acceptability by using descriptive analysis with scoring method. The evaluation sheet is shown in Appendix C2. The score for each attribute ranges from 1 to 7 (Table 3.4).

Table 3.4 Definition of scores for color, odor, smoothness, viscosity and acceptability

Score	Color	Odor	Smoothness	Viscosity	Acceptability
1	Light Yellow	Odorless	Extremely smooth	Not viscous	Not acceptable
2	Dark Yellow	Very mild garlic odor	Highly smooth	Very low viscosity	Quite acceptable
3	Very dark Yellow	Mild garlic odor	Very smooth	Low viscosity	Rather acceptable
4	Light brown	Moderately strong garlic odor	Smooth	Moderately viscous	acceptable
5	Brown	Strong garlic odor	Rather smooth	Rather viscous	Very acceptable
6	Dark brown	Very strong garlic odor	Quite smooth	Highly viscous	Highly acceptable
7	Very dark brown	Extremely strong garlic odor	Not smooth	Extremely viscous	Extremely acceptable

3.2.8.4 Shelf life determination

The salad dressing (100 g) was mixed with garlic oil microcapsules (3.962 g) using a blender at low speed until it was homogeneous. The control sample was salad dressing that was not added with garlic oil microcapsules. The samples were stored at room temperatures (~25˚C) for up to 7 days. Samples were immediately taken with a micropipette at the initial time (0 day) and at an interval of two days to determine for microorganisms load by plate count method as described in Appendix B2. The samples were taken to measure for color and sensory assessment following 3.2.8.2 and 3.2.8.3.

3.2.9 Statistical analysis

The statistical analysis of the results was conducted by the analysis of variance (ANOVA) and Duncan's New Multiple Range Test to evaluate the difference between means at 95% confidence interval.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Critical micelle concentration (CMC) of Tween® in maltodextrin solutions

In order to determine the critical micelle concentration (CMC), the surface tension of all Tween®20 and 80 in 20 g/dL maltodextrin solutions was measured after preparation. CMC is a minimal concentration of emulsifier which micelles are spontaneously formed in solution leading to dispersing of oil (hydrophobic) into an aqueous phase (hydrophilic). In general, developed antimicrobial products consist of both water soluble and non-water soluble extract. Emulsifier enables non-water soluble extracts or oils to disperse throughout a water base to form a cream or an emulsion. The emulsifier concentration had to be above its CMC value. In this study, the CMC value was determined from association between surface tensions of emulsifier solutions and emulsifier concentrations. The surface tension of all diluted Tween®20 and 80 (0-5.11% by weight) in 20 g/dL maltodextrin solution was measured by the pendant drop method. Figure 4.1 showed the CMC values of Tween®20 and 80 that were identified by the intersection of the two straight lines tangent to different slopes. The CMC was 0.49 %w/w for both Tween®20 and 80. The surface tension at CMC was 36.9 and 41.96 $dyn \cdot cm^{-1}$ for Tween®20 and 80, respectively (Table 4.1).

Table 4.1 Critical micelle concentrations and surface tensions of Tween®20 and Tween®80 determined by the pendant drop method.

Continuous phase	CMC* (%w/w)		ST* ($dyn \cdot cm^{-1}$)	
	Tween®20	Tween®80	Tween®20	Tween®80
20 $g \cdot dL^{-1}$ Maltodextrin solution	0.49±0.01	0.49±0.01	36.69±0.48	41.96±0.02

*CMC: Critical Micelle Concentration, ST: Serface tension ($dyn \cdot cm^{-1}$)

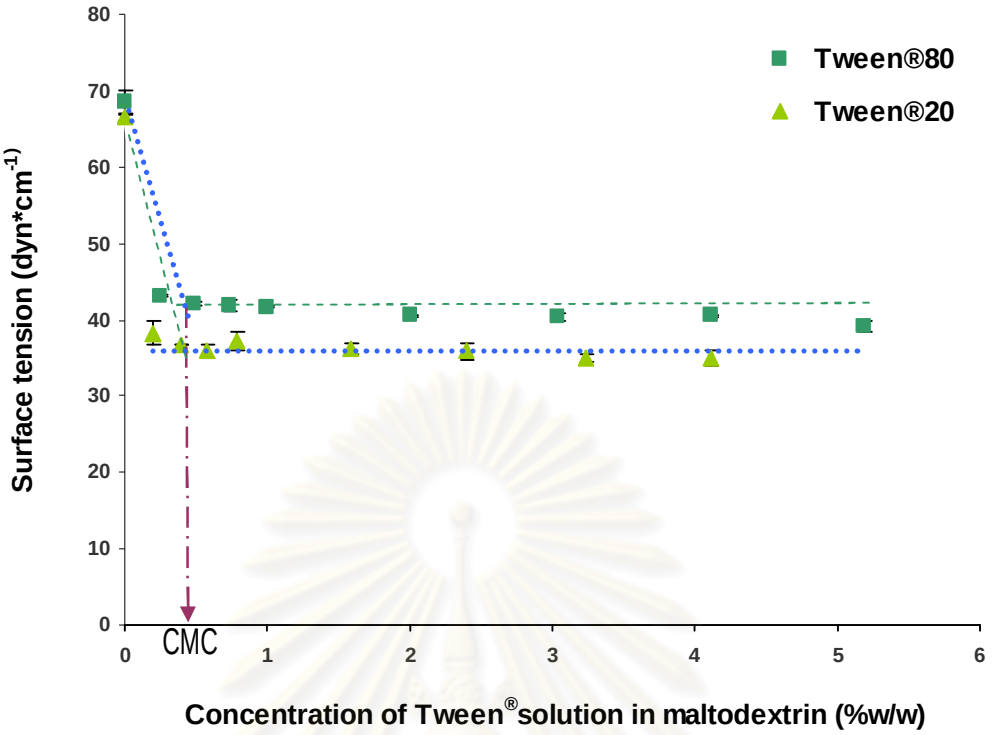


Figure 4.1 Interfacial tensions of Tween®20 and Tween®80 in 20 g/dL in maltodextrin solution.

The surface tension at CMC of Tween®20 (36.69±0.48 dyn·cm^{-1}) was lower than that of Tween®80 (41.96±0.02 dyn·cm^{-1}). This is due to the fact that the HLB (Hydrophilic-Lipophilic Balance) value of Tween®20 which is 16.7 is higher than that of Tween®80 which is 15.0 (Malgorzata et al., 2007). Tween® is a non-ionic surfactant that could be absorbed on an oil droplet surface and aggregate its molecules to produce micelle. Generally, water solubility of Tween® depends on the type of fatty acid in the ester group of sorbitan and the amount of oxyethylene groups in polyethylene glycol structure. Fewer oxyethylene compounds results in better solubility in oil compared to that in water. At a concentration above its CMC, an emulsifier could aid in the production of a stable emulsion. Therefore, 0.6%, 0.8% and 1% w/w of Tween®20 were selected to prepare an emulsion in subsequent studies.

4.2 Stability of garlic oil in emulsions

All resulting oil-in-water (O/W) emulsion appeared milky white and had low viscosity. No separation of oil was observed after preparation. The emulsion tended to form rapidly at low oil to maltodextrin ratios and high Tween®20 concentration.

4.2.1 Emulsion stability

All prepared emulsions were stored at room temperature (~25^{o}C) for 48 hours. The results showed that there was no separation of oil phase in both garlic oil emulsions with maltodextrin and those without maltodextrin. Emulsions containing maltodextrin provided solutions with precipitate in the bottom layer (Figure 4.2) whereas those without maltodextrin maintained milky white solution (Figure 4.3). However, all emulsions without maltodextrin prepared by using homogenizing rotational speed of 13,000 rpm consisted of two separate layers having different color (Figure 4.3(1)).

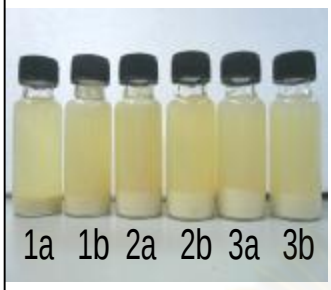
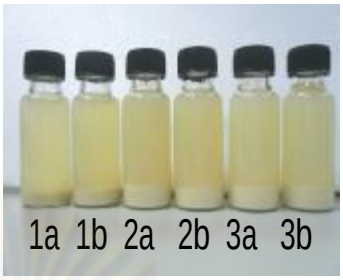
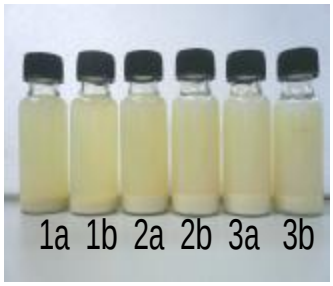
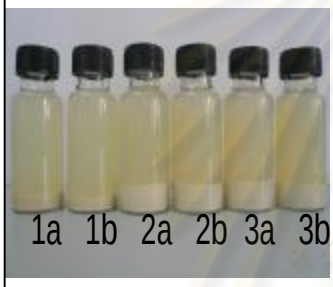
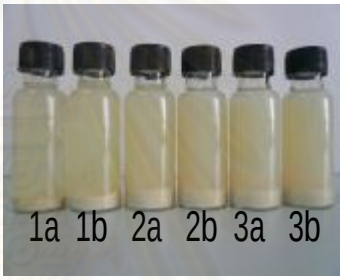
Tween 20	Oil to maltodextrin ratio		
	0.1:1	0.15:1	0.2:1
0.6% w/w			
0.8% w/w			
1% w/w			

Figure 4.2 Emulsions of garlic oil (oil-to-maltodextrin ratio of 0.1:1, 0.15:1, and 0.2:1) in 0.6%, 0.8% and 1% w/w Tween®20 in 20 g/dL maltodextrin prepared at homogenizing rotational speed of 13,000 rpm (1), 19,000 rpm (2) and 24,000 rpm (3) for 5 min (a) and 10 min (b) and stored at 25°C for 48 hours.

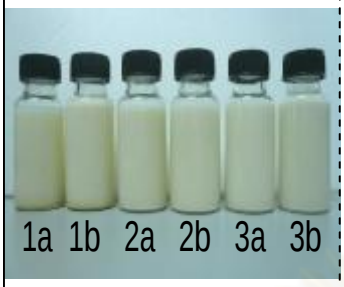
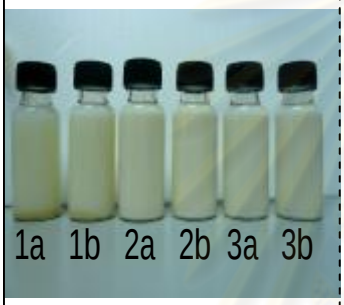
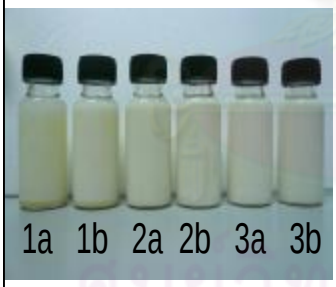
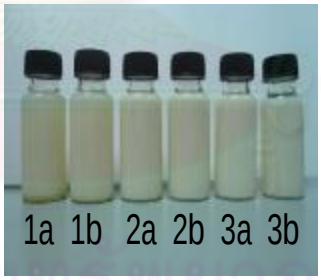
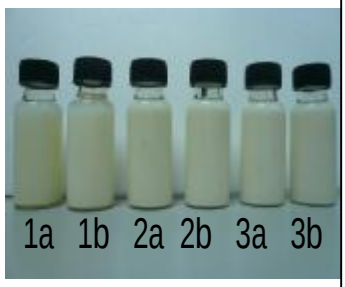
Tween 20	Oil ratio		
	0.1:1	0.15:1	0.2:1
0.6% w/w			
0.8% w/w			
1% w/w			

Figure 4.3 Emulsions of garlic oil (2%, 3% and 4% w/w) in 0.6%, 0.8% and 1% w/w Tween®20 in distilled water prepared at homogenizing rotational speed of 13,000 rpm (1), 19,000 rpm (2) and 24,000 rpm (3) for 5 min (a) and 10 min (b) and stored at 25°C for 48 hours.

From the results, this emulsion system consisted of garlic oil dispersing in maltodextrin (DE=10) solution was stabilized by Tween®20 which acted as an emulsifier. Tween®20 (polyoxyethylene sorbitan monolaurate) is a non-ionic surfactant. The surface active molecules of Tween®20 absorb to the surface of oil droplets during homogenization and protect them from coming close enough to aggregate (Weiss et al., 1996; Krstonosic, 2009).

In aqueous phase, maltodextrin plays a major role as a hydrocolloid stabilizer in increasing small viscosity of continuous phase of an emulsion, which leads to slowing down the gravitational separation of the droplets (Dickinson, 2003). When maltodextrin is dissolved in water, many hydroxyl groups (-OH) of their molecules can hydrate the water continuously by hydrogen bonding until complete solvation. Although 98% of the maltodextrin is soluble in water, there is about 2% non-water soluble fraction precipitating in solution. Therefore, observable precipitate in the bottom of O/W emulsions prepared in maltodextrin solution could be expected. Lower available water resulting from emulsifier-water interaction leads to an increase in precipitation of maltodextrin as well.

At low homogenizing rotational speed of 13,000 rpm, emulsion without maltodextrin showed separation in two different color layers within 24 hours. The bottom layer had darker milky color compared to the top layer. This resulted from different droplet sizes between layers due to flocculation of droplets. Flocculation of droplets which resulted in larger oil droplet size had a higher density than small droplets, thus sank to the bottom layer.

4.2.2 Garlic oil droplet size

Average size D[3,2] of garlic oil droplet in emulsion with various Tween®20 concentrations (0.6%, 0.8%, and 1%) and 20 g/dL matodextrin solution are compared in Figure 4.4. Range of average oil droplet sizes was 0.126 to 0.281 μm. It could be observed that an increase in homogenizing rotational speed from 13,000 to 19,000 and 13,000 to 24,000 rpm led to a significant decrease ($p \leq 0.05$) of oil droplet sizes in emulsion prepared with 0.6% and 0.8% w/w Tween®20. For emulsion with 1% w/w Tween®20, oil droplet size significantly decreased ($p \leq 0.05$) when the rotational speed increased in every level (13,000 to 19,000 rpm, 13,000 to 24,000 rpm and 19,000 to 24,000 rpm) (Figure 4.4). Higher homogenizing rotational speed, hence higher power input, caused oil droplet size to become smaller. On the contrary, homogenizing time did not influence the oil droplet size of emulsions with 0.6% and 0.8% w/w Tween®20 concentrations. But it still affected the droplet size of emulsions with 1%Tween®20 by decreasing significantly the size of oil droplets in emulsion prepared with 0.15:1 oil-to-maltodextrin ratio with 24,000 rpm homogenizing speed from 5 to 10 minutes (Figure 4.4). For the effect of oil-to-maltodextrin ratio, droplet sizes of oil in the emulsions prepared with different ratios (0.1:1, 0.15:1 and 0.2:1) were significantly different ($p \leq 0.05$). Higher oil-to-maltodextrin ratio tended to yield larger oil droplet size for all emulsions. The effect was more pronounced when higher homogenizing rotational speeds (19,000 and 24,000 rpm) were employed.

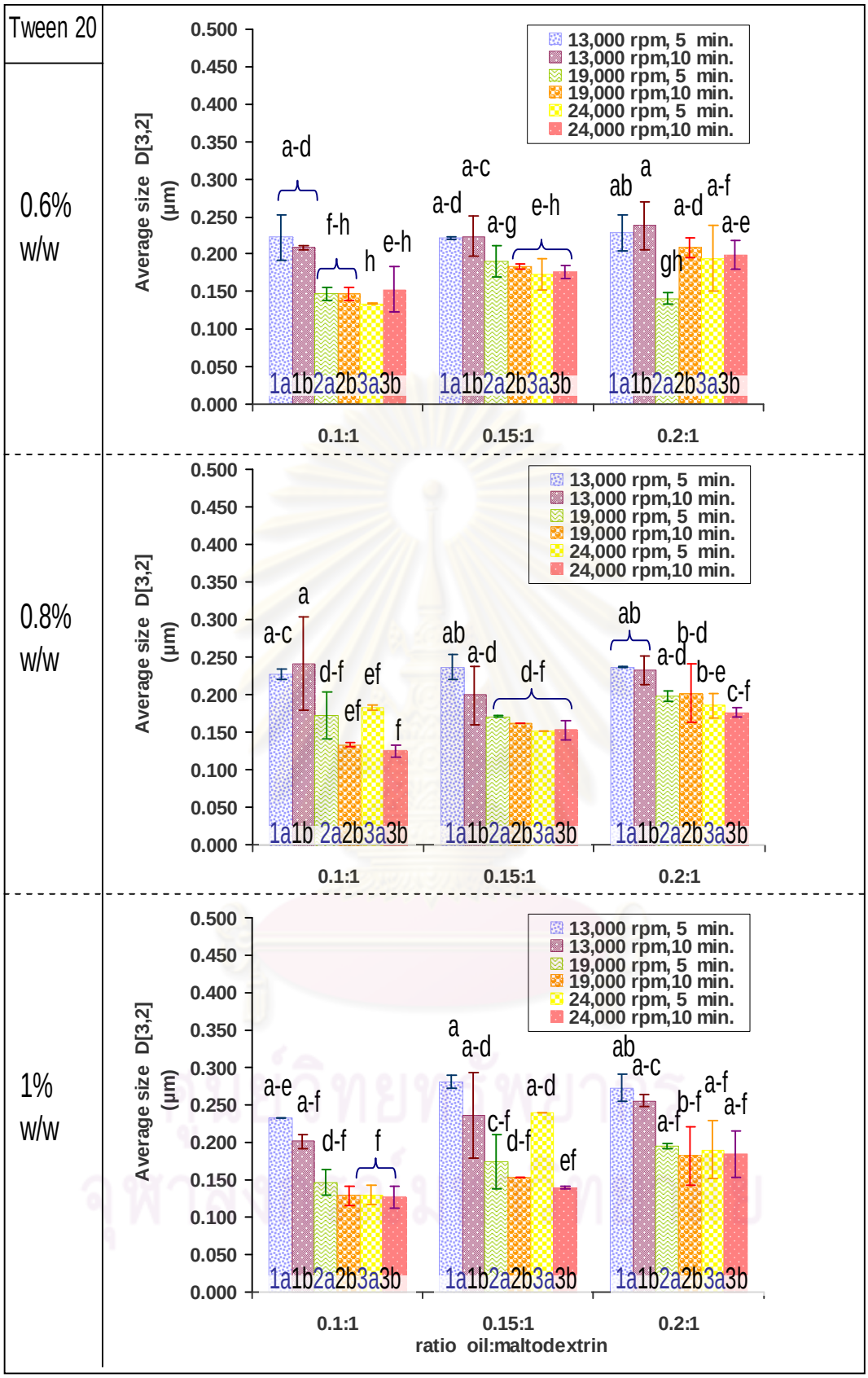


Figure 4.4 Droplet size of garlic oil emulsion in various Tween®20 concentrations in 20 g/dL maltodextrin solution prepared by homogenizing at 13000 (1), 19000 (2) and 24,000 (3) rpm for 5 (a) and 10 (b) min stored at 4°C for 24 hours.

a, b, c, d, e, f Different letters above bars of each group denote significant difference ($p \leq 0.05$)

The increase in rotational speed from 13,000 rpm to 19,000 rpm and 24,000 rpm also decreased the droplet size from 0.223 to 0.153 μm, 0.222 to 0.176 μm and 0.229 to 0.200 μm for 0.1:1, 0.15:1 and 0.2:1 oil to maltodextrin ratio in 0.6% w/w Tween®20, and from 0.227 to 0.126 μm, 0.237 to 0.153 μm and 0.237 to 0.176 μm for 0.1:1, 0.15:1 and 0.2:1 oil to maltodextrin ratio in 0.8 %w/w Tween®20, and from 0.232 to 0.127 μm, 0.281 to 0.140 μm and 0.273 to 0.185 μm for 0.1:1, 0.15:1 and 0.2:1 oil to maltodextrin ratio in 1 %w/w Tween®20, respectively, as the increase of homogenizer speed led to the development of high shear stress, thus increase the interfacial area and reduce the size of oil droplets. McClements (2005) reported that the size of droplet in an emulsion could be reduced by increasing the amount of energy supplied during homogenization. The increase of rotational speed and the length of time to blend the sample can increase the energy input. When considering the influence of maltodextrin addition on garlic oil droplet size, the results showed that, at every Tween®20 concentrations, the oil droplet size in emulsions with and without maltodextrin did not differ significantly ($p>0.05$) and varied in the oil droplet size range 0.111 to 0.260 μm (Figures 4.5, 4.6 and 4.7). This is because maltodextrin is not particularly surface-active and is also water soluble (Dokic et al., 1997). In an emulsion system, the major part of maltodextrins (~98%) was dissolved and the stabilizing action in O/W emulsion comes from small viscosity modification or film formation on micelle of the aqueous continuous phase surrounding the oil droplet (Dickinson, 2006).

The emulsion size may be an important parameter determining the stability of initial feed emulsion and may also affect the characteristic of final spray-dried microencapsulation powder.

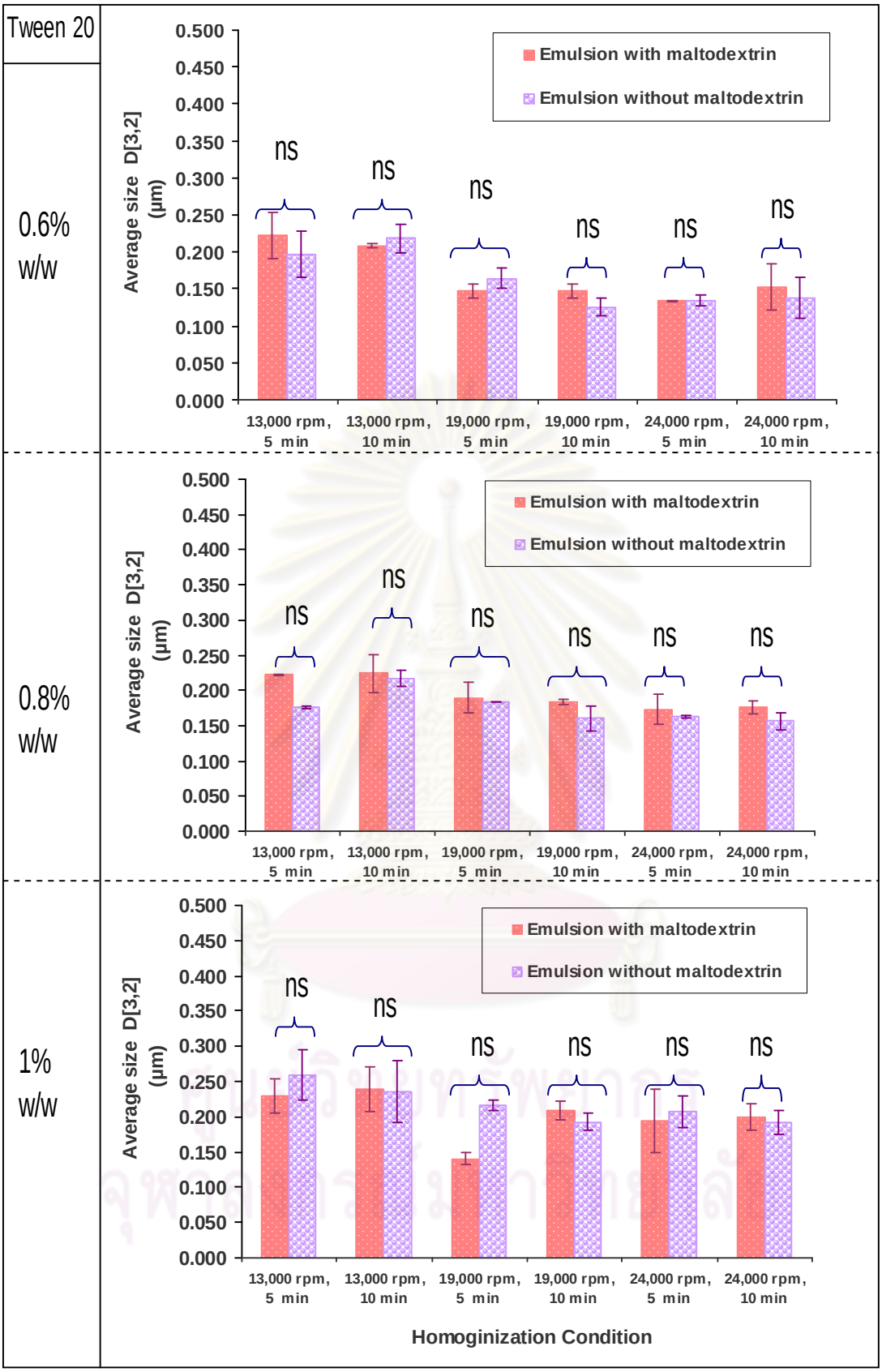


Figure 4.5 Droplet size; D[3,2], of garlic oil emulsion containing 0.6% w/w Tween®20 at 0.1:1 (A), 0.15:1 (B), and 0.2:1(C) oil-to-maltodextrin ratio stored at 4°C for 24 hours. ns above bars of each group denotes no significant difference ($p>0.05$).

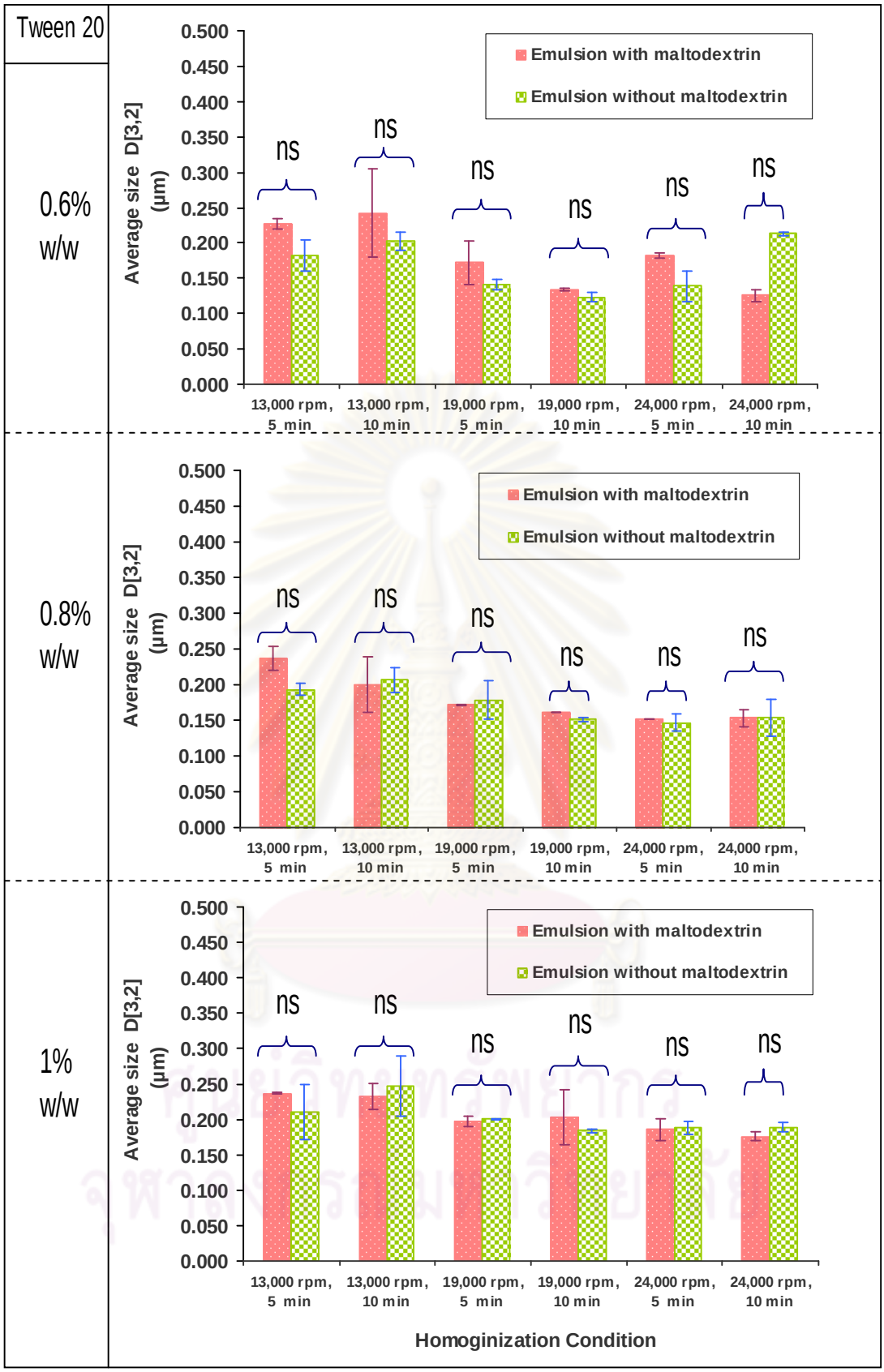


Figure 4.6 Droplet size; D[3,2], of garlic oil emulsion containing 0.8% w/w Tween®20 at 0.1:1 (A), 0.15:1 (B), and 0.2:1 (C) oil-to-maltodextrin ratio stored at 4°C for 24 hours. ns above bars of each group denotes no significant difference ($p>0.05$)

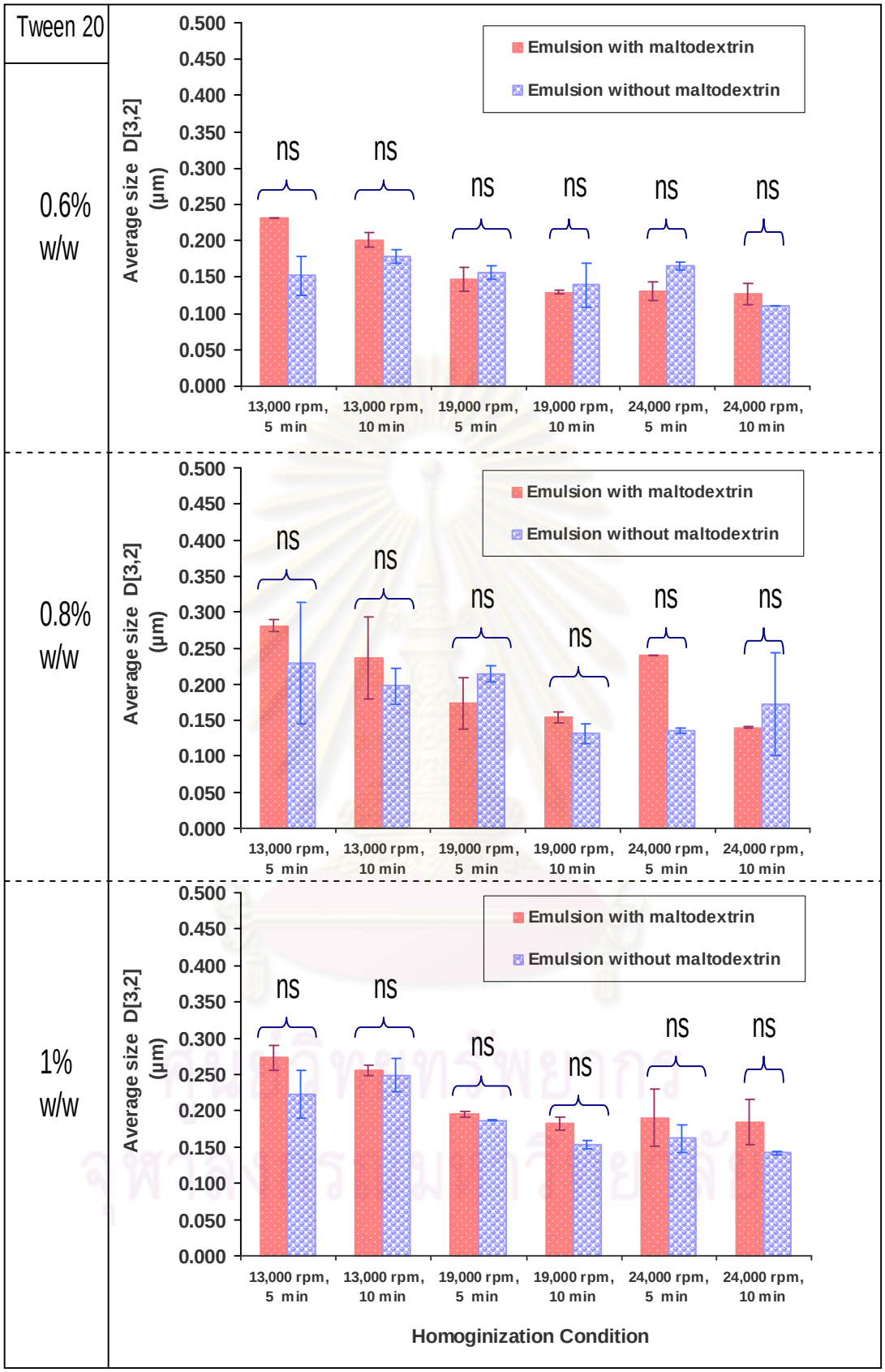


Figure 4.7 Droplet size; D[3,2], of garlic oil emulsion containing 1% w/w Tween®20 at 0.1:1 (A), 0.15:1 (B), and 0.2:1 (C) oil to maltodextrin ratio stored at 4°C for 24 hours. ns above bars of each group denotes no significant difference ($p>0.05$).

For initial feed emulsion, the optimal proportion of preparation could be selected from the preparation providing the smallest oil droplet size and more oil ratio to spray drying. Several studies (Risch and Reinneccius, 1988; Re', 1998) have reported that an emulsion with smaller droplet size yields higher oil retention percentage in the dried powders leading to better ability to keep the core material in a product for a longer period of time. From the results, smaller oil droplet sizes were observed at 19,000 rpm and 24,000 rpm rotational speed of homogenization at various Tween®20 concentrations (0.6%, 0.8% and 1%w/w). Due to more efficient oil retention during microencapsulation process, 0.2:1 oil to maltodextrin ratio was selected. At 0.2:1 oil-to-maltodextrin ratio, there were no significant differences between oil droplet sizes of the emulsions homogenized at 19,000 (0.111 to 0.260 μm) and 24,000 (0.141 to 0.273 μm) rpm rotational speed, and time of homogenization of at various Tween®20 concentrations. Therefore, the optimal preparation condition for feed emulsion was 19,000 rpm rotational speed for 5 minutes.

4.3 Physical and chemical properties of spray dried microcapsules

Three selected emulsion feeds containing 0.6%, 0.8% and 1% w/w Tween®20 and 20 g/dL maltodextrin with 0.2:1 oil-to-maltodextrin were spray dried at various inlet air temperatures(120, 160, 180 and 200°C) at a pressure of 3 bars, and a feed rate of 25±5 mL/minute. After the spray drying process, all emulsion feeds yielded dried powder with light yellow color (Figure 4.8).

Figure 4.8 Spray-dried garlic oil microcapsules.

4.3.1 Powder morphology and particle size analysis

The outer topographies of spray-dried microencapsulated products were assessed by SEM (Figures 4.9 to 4.12). The spray-dried powders prepared at different inlet air temperatures (120±5°C, 160±5°C, 180±5°C and 200±5°C) were almost spherical and had small holes dispersing on their wall. The particles appeared in either smooth or rough surface. At inlet temperature of 120°C, the particle surface was rough. As the temperature was increased from 120°C to 160°C, 180°C and 200°C, the surface became smoother for each Tween®20 concentration (0.6, 0.8 and 1% w/w). When the microcapsules were cross-sectioned, it could be observed that the core material was in the form of small droplets embedded in the wall matrix layer. In the center of the capsules, a large void could also be observed which occupied most of the capsule volume. This structure is called microsphere structure (Figure 4.9, 4.10, 4.11 and 4.12) and is the particle that core materials spread continuously in the wall layer.

The large void in the center of a microcapsule is called balloon, which resulted from the steam formed in the interior of the drying droplet owing to direct contact with high inlet air temperature. This caused the droplet to puff or balloon that led to a thin-walled hollow particle (Jafari et al., 2008).

A low inlet temperature, the particle surface dents were formed by shrinkage of the particles during drying and cooling, similar dents were observed in the study on milk powder (Rosenberg et al., 1985). Sheu et al. (1998) spray-dried microcapsules with wall material consisting of whey protein isolate, maltodextrin (DE=5, 10 and 15) and corn syrup solids (DE=24) which showed notable surface indentations, due to their wall composition, atomization and drying parameter. They have also reported the morphological variations (size, structure and appearance), the beginning spherical droplet could form particles with irregular surface in many cases owing to internal formation of voids and dents, depression and external fracture.

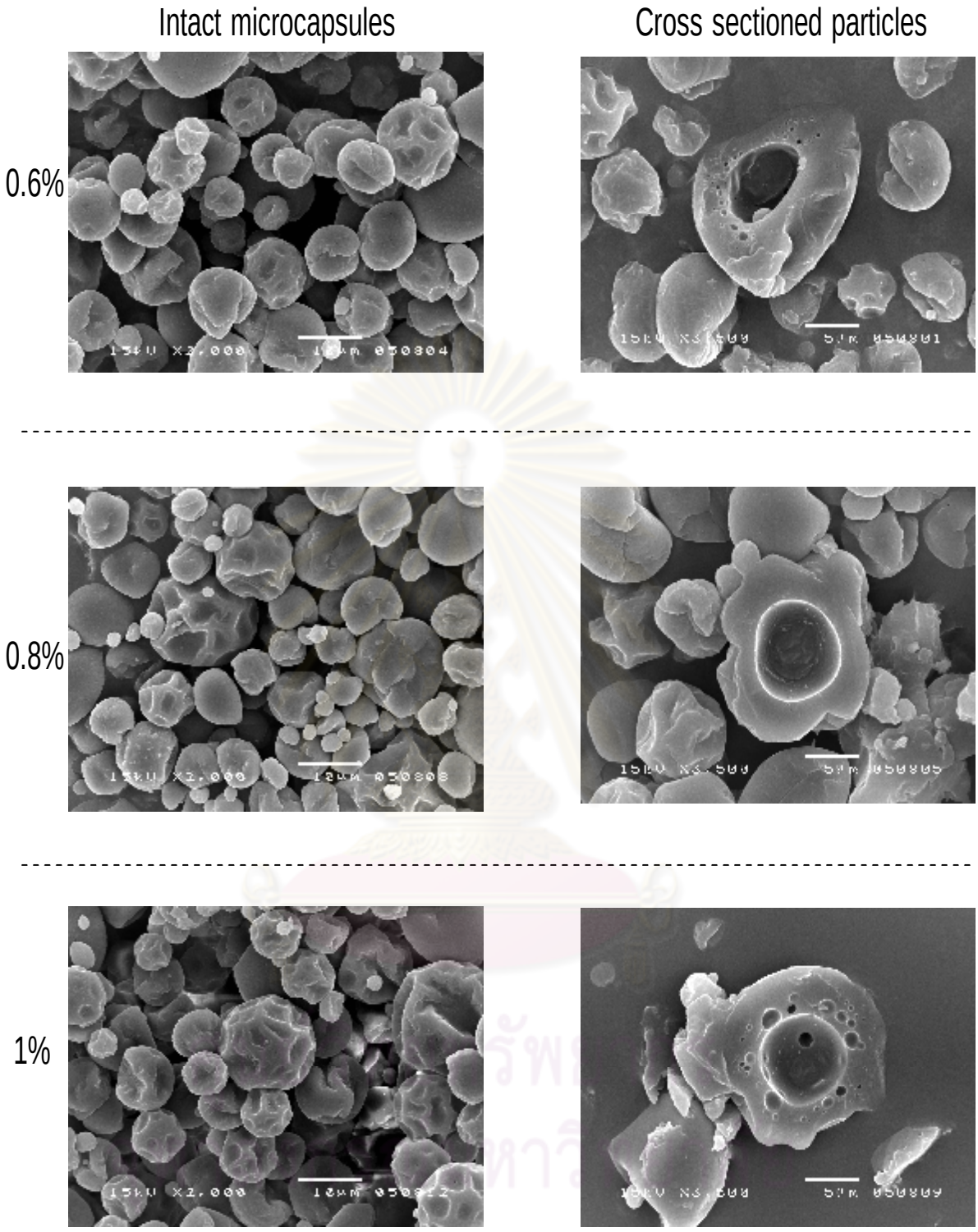


Figure 4.9 SEM images of spray-dried garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil to maltodextrin ratio and 0.6%, 0.8% and 1% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 120±5°C.

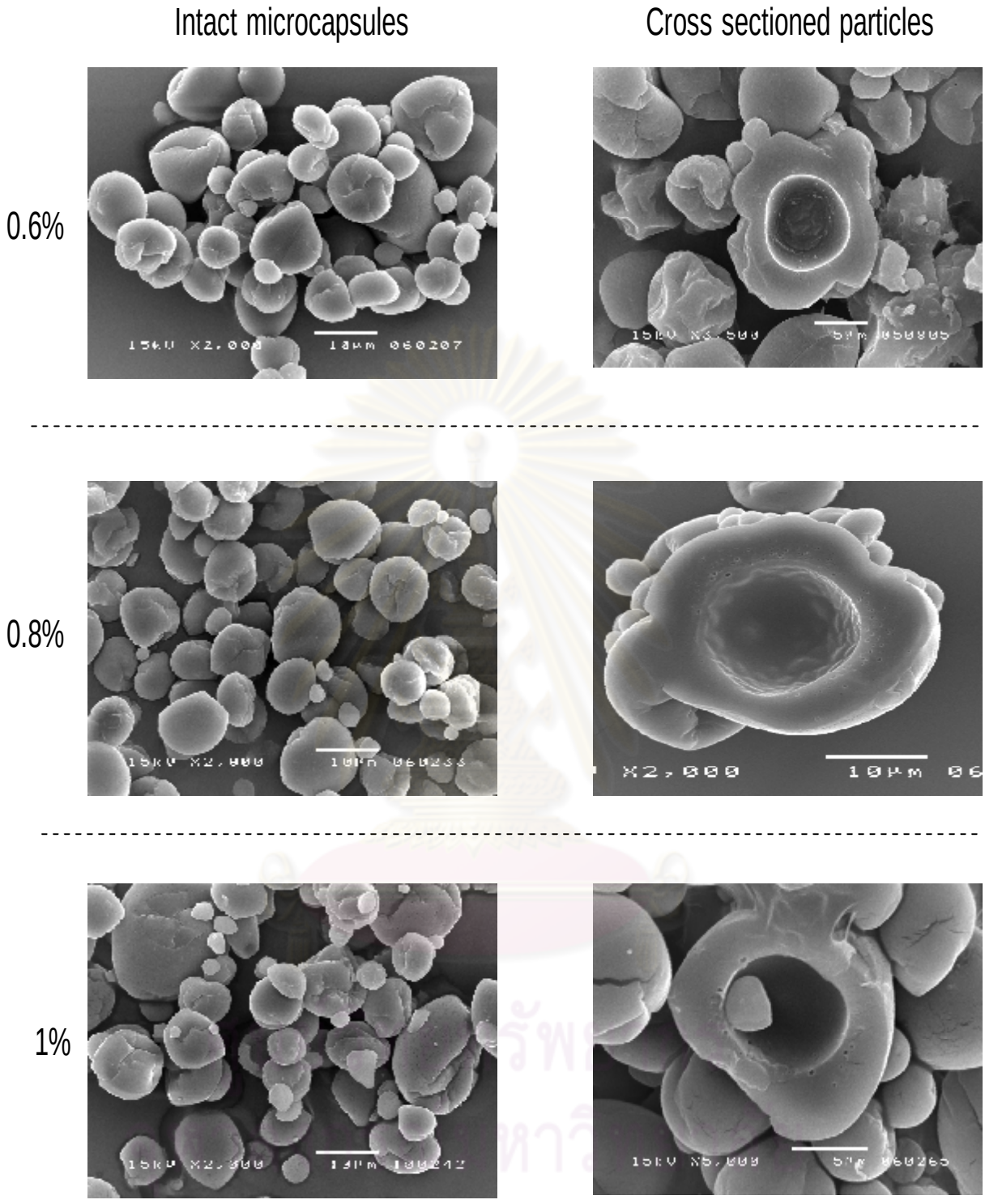


Figure 4.10 SEM images of spray-dried garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil to maltodextrin ratio and 0.6%, 0.8% and 1% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 160±5°C.

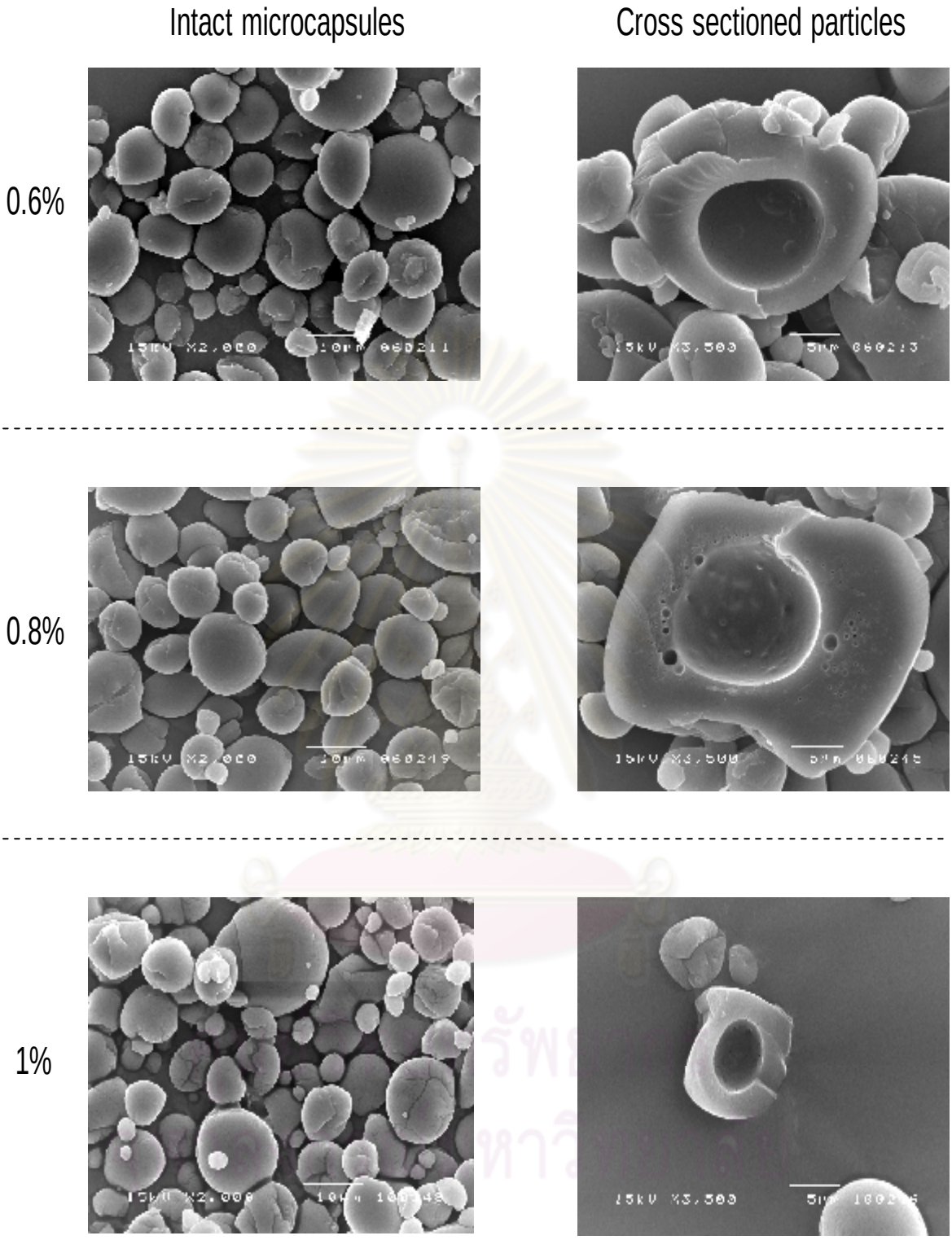


Figure 4.11 SEM images of spray-dried garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil to maltodextrin ratio and 0.6%, 0.8% and 1% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 180±5°C.

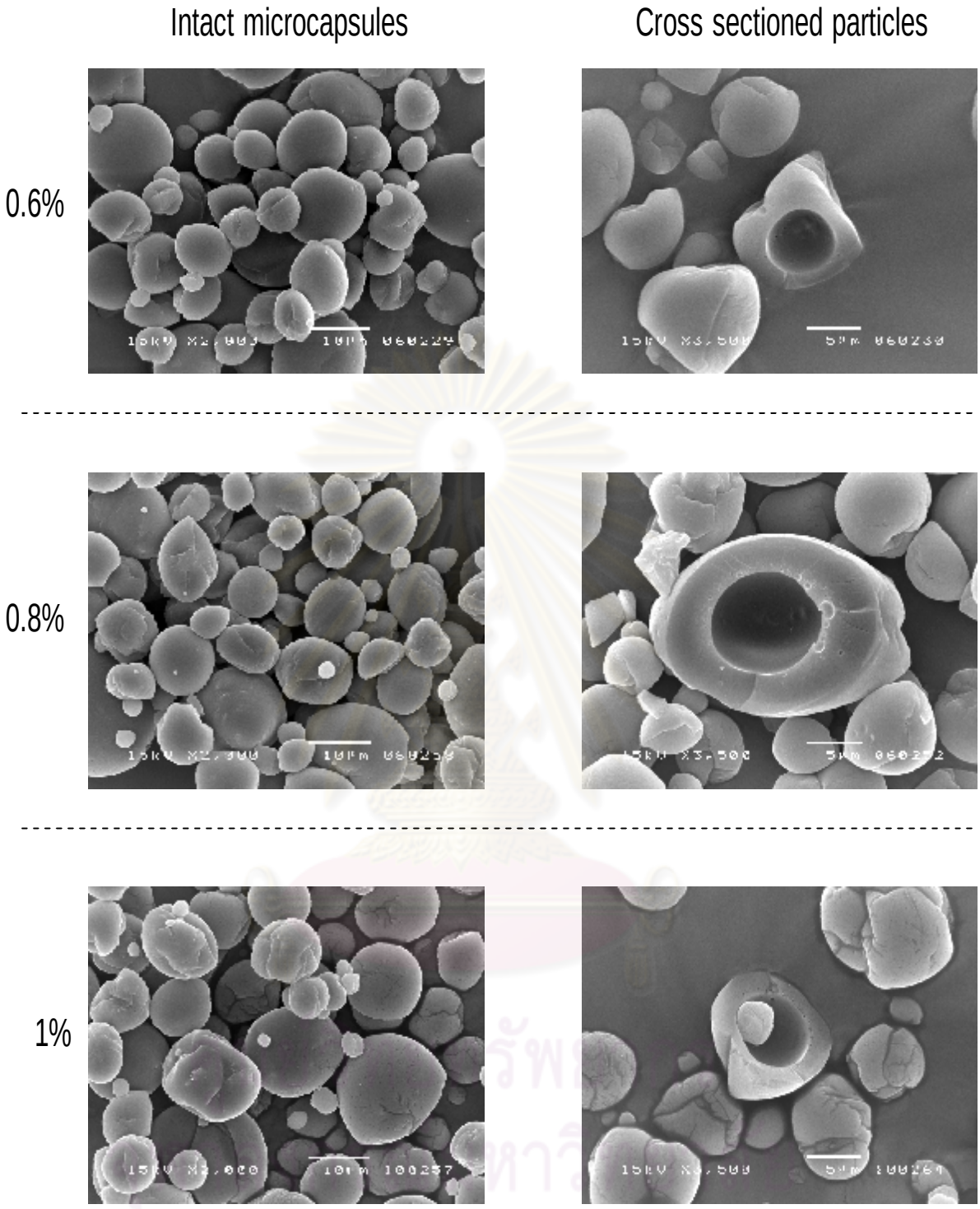


Figure 4.12 SEM images of spray-dried garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil to maltodextrin ratio and 0.6%, 0.8% and 1% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 200±5°C.

The size distribution of garlic oil microcapsules was determined by using a SemAfore program (Version 4.01). The results were expressed as the surface area moment mean D[3,2] or Sauter mean diameter (SMD) of microcapsules. The surface area moment mean of the microcapsules preparared from garlic oil of 0.2:1 oil to maltodextrin with 0.6%, 0.8% and 1% w/w Tween®20 in 20 g/dL maltodextrin ranged 9-13 μm, 9-12 μm, and 10-13 μm, respectively. According to Table 4.2, the average size of microcapsules spray-dried at 120°C inlet temperature had the largest size for every Tween®20 concentration. There was no significantly difference ($p>0.05$) between the size of microcapsules spray-dried at 180 and 200°C inlet temperature for both 0.6% and 0.8% w/w Tween®20 concentrations. The microcapsules prepared at 160 and 180°C for both 0.8% and 1% Tween®20 had a size ranges (9.62, 9.82, 10.38 and 10.46 μm) that were not significant different. The largest average size of microcapsules spray-dried at 120°C might result from their rough surface that caused higher length of diameter and more surface area.

The parameters such as inlet air temperature, concentration of polymeric solution have an impact on microcapsule size (Newton, 1966). The effect of air inlet temperature on size associates with kind of coating materials that have different structures. In addition, Newton (1966) reported that particle size of some material increased with increasing drying air temperature. The previous studied (Soottitantawat et al., 2004) reported that the large powder size showed the higher and lower release of encapsulated oil than small powder size during storage. However, it should consider other parameters such as moisture and kind of wall materials as well.

Table 4.2 Size of spray-dried garlic oil microcapsules (0.2:1 oil to maltodextrin ratio) at various Tween®20 concentrations (0.6%, 0.8% and 1% w/w) in 20 g/dL maltodextrin spray-dried at various temperature (120, 160, 180 and 200°C) in a feed rate of 25 mL·min^{-1} and pressure of 3 bars.

Emulsion System	Temperature of spray drying		Average diameter* of microcapsules (μm)	The surface area moment mean* of microcapsules (μm)
	Inlet temperature (°C)	Outlet Temperature (°C)		
Garlic oil in 0.6% Tween® 20 in 20 g/dL Maltodextrin	120±5	70±5	10.21^{a}±0.21	13.07^{a}±0.22
	160±5	75±5	9.28^{c}±0.18	11.28^{bc}±1.18
	180±5	80±5	8.63^{de}±0.11	9.82^{d}±0.01
	200±5	85±5	7.81^{f}±0.40	9.81^{d}±0.94
Garlic oil in 0.8% Tween® 20 in 20 g/dL Maltodextrin	120±5	70±5	9.98^{ab}±0.16	11.98^{ab}±0.82
	160±5	75±5	8.40^{ef}±0.19	9.62^{d}±0.11
	180±5	80±5	8.44^{ef}±0.01	9.82^{d}±0.13
	200±5	85±5	9.05^{cd}±0.25	10.19^{cd}±0.66
Garlic oil in 1%Tween®20 in 20 g/dL Maltodextrin	120±5	70±5	9.54^{bc}±0.48	12.03^{ab}±0.18
	160±5	75±5	9.13^{cd}±0.38	10.38^{cd}±0.57
	180±5	80±5	9.03^{cd}±0.89	10.46^{cd}±0.33
	200±5	85±5	9.50^{bc}±0.33	11.94^{ab}±0.76

$^{a, b, c, d, e, f}$ Different letters in the same column denote significant difference ($p \leq 0.05$)

* Average of 50 particles

4.3.2 Bulk density, moisture content, water activity, solubility and total oil content

Table 4.3 shows the physical and chemical properties of spray-dried microcapsules including bulk density, moisture content, water activity, solubility and total oil content. The bulk densities of all spray-dried microcapsule samples were not significantly different ($p>0.05$) and they ranged from 0.38 to 0.54 g/cm^3.

The moisture content of the sample spray-dried at 120°C inlet temperature for any Tween®20 concentration was significantly different ($p \leq 0.05$) from the sample spray-dried at higher inlet air temperatures. This result also related with the water activity of the sample prepared at the inlet temperature of 120°C. All garlic oil microcapsules possessed low percentage of oil which oil retention decreased with the inlet temperature increased. Especially, the microcapsules prepared from the emulsion containing 0.6% and 0.8% w/w Tween®20 and spray-dried at an inlet temperature of 180°C obtained significantly higher oil content than microcapsules spray-dried at other inlet temperatures. However, it could be observed that there were no significant differences ($p > 0.05$) in microcapsule solubility.

Table 4.3 Properties of spray-dried garlic oil microcapsules (0.2:1 oil-to-maltodextrin ratio) at various Tween®20 concentrations (0.6%, 0.8% and 1% w/w) in 20 g/dL maltodextrin spray-dried at various temperature (120, 160, 180 and 200°C) in a feed rate of 25 mL·min^{-1} and pressure of 3 bars.

Sample*	Inlet Temp. (°C)	The physical and chemical properties of the spray-dried powder*					
		Moisture Content (% w/w)	Water activity (A_w)	Total oil content (% w/w)	Encapsulation efficiency (%)	Bulk density^{ns} (g/cm^3)	Solubility test^{ns} (% w/w)
0.6% Tween20	120±5	2.06^{a} ±0.18	0.26^{a} ±0.02	1.82^{b} ±0.72	9.09	0.53±0.01	95.74
	160±5	0.52^{b} ±0.01	0.13^{cde}±0.01	2.21^{ab}±0.05	11.10	0.38±0.16	96.72
	180±5	0.64^{b} ±0.13	0.10^{def}±0.01	3.69^{a} ±1.68	18.45	0.51±0.01	96.92
	200±5	0.54^{b} ±0.13	0.11^{def}±0.03	2.84^{ab}±0.90	8.37	0.51±0.01	94.94
0.8% Tween20	120±5	1.87^{a} ±0.70	0.17^{bc}±0.05	1.34^{b} ±0.16	6.72	0.54±0.02	96.07
	160±5	0.37^{b} ±0.07	0.11^{def}±0.01	1.76^{b} ±0.21	8.80	0.50±0.01	96.12
	180±5	0.39^{b} ±0.12	0.08^{f} ±0.01	3.68^{a} ±0.93	18.40	0.42±0.16	97.08
	200±5	0.27^{b} ±0.01	0.08^{def}±0.01	2.57^{ab}±0.45	12.85	0.52±0.01	96.05
1% Tween20	120±5	2.03^{a} ±1.08	0.18^{b} ±0.04	2.29^{b} ±0.15	11.45	0.52±0.01	97.01
	160±5	0.51^{b} ±0.12	0.12^{def}±0.01	1.67^{b} ±0.59	8.35	0.50±0.01	97.38
	180±5	0.53^{b} ±0.02	0.15^{bcd}±0.01	2.02^{b} ±0.04	10.1	0.49±0.01	96.52
	200±5	0.10^{b} ±0.06	0.10^{def} ±0.00	1.99^{b} ±0.07	9.99	0.48±0.01	97.79

* Tween®20 in 20 g/dL maltodextrin solution with 0.2:1 garlic oil to maltodextrin

^{a,b,c,d,e,f} Different letters in the same column denote significant difference ($p \leq 0.05$)

^{ns} no significant difference ($p > 0.05$)

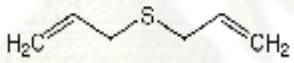
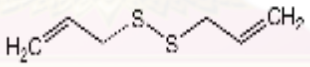
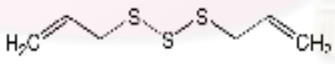
When the inlet temperature increased from 120°C to 200°C, the moisture content and water activity (a_w) of the microcapsules decreased. The moisture content decreased from 2.06% to 0.54% w/w, 1.87% to 0.27% w/w μm and 2.03% to 0.10% w/w for microcapsules prepared in 0.6%, 0.8% and 1% w/w Tween®20, respectively. Water activity (a_w) decreased from 0.26 to 0.11, 0.17 to 0.08 and 0.18 to 0.10 for microcapsules prepared in 0.6%, 0.8% and 1% w/w Tween®20, respectively. This was due to higher evaporation rate. In many cases, increasing the air inlet temperature increases the rate of film formation and volatiles retention because the crust layer forms immediately from a rapid drying rate (Anker and Reinneccius, 1988; Bhandari et al., 1992; Re' et al., 1998). However, Zakarian and King (1982) reported that a high inlet air temperature resulted in an excessive evaporation leading to cracks in the particle wall that induced a loss of volatiles ingredient. From Table 4.3, at the inlet temperatures of 160°C and 180°C, the total garlic oil tended to increase 2.21% to 3.69 % w/w, 1.76% to 3.68 % w/w and 1.67% to 2.62 % w/w for microcapsules prepared in 0.6%, 0.8% and 1% w/w Tween®20, respectively. On the other hand, at the inlet temperatures of 180°C and 200°C, a decrease in total oil 3.69% to 2.84% w/w, 3.68% to 2.57% w/w and 2.02% to 1.99% w/w for microcapsules prepared in 0.6%, 0.8% and 1% w/w Tween®20, respectively, was observed. It was possible that when the inlet temperature reached 200°C, too rapid evaporation caused dried particles to crack leading to the loss of garlic oil. The lowest oil retention resulted at 120°C inlet air temperature due to low evaporation rate that caused slower film formation rate around oil droplets in the drying process. As a consequence, loss of volatile substances was observed. The highest garlic oil retention was microcapsules prepared 0.6% w/w Tween®20 in 20 g/dL maltodextrin and spray dried at 180°C.

The results showed that there was no significantly effect of Tween®20 concentration (0.6%, 0.8% and 1% w/w) and Inlet air temperature (120°C, 160°C, 180°C and 200°C) on difference of bulk density and solubility of microcapsules.

4.3.3 Bioactive compound

Gas chromatography (GC) was used for detection and quantification of main antimicrobial compounds in garlic oil and microcapsules. According to Ross et al. (2000) diallyl disulfides (DADS) and diallyl trisulfides (DATS) are an indicator for antimicrobial ability of garlic oil. From Table 4.4, four major compounds including diallyl disulfides (DADS), diallyl trisulfides (DATS), diallyl sulfides (DAS) and cyclic sulfurs (S_8) were detected in garlic oil. GC analysis shows the content of the four compounds that found in garlic oil. Garlic oil contained 1.29% of DADS and 1.17% of DAS which were higher than DATS (0.56%) and S_8 (0.21%).

Table 4.4 Concentration of bioactive compounds found in garlic oil

Sulfur compounds	Molecular structure*	Concentration (% w/w)
DAS		1.17
DADS		1.29
DATS		0.56
S_8		0.21
Total		3.23

Note: DAS = Diallyl sulfides, DADS = Diallyl disulfides, DATS = Diallyl trisulfides, S_8 = Cyclic sulfurs

* Source: Jirovetz (1992) and Kimbaris et al. (2005)

From Table 4.4, the amount of sulfur compounds in garlic oil is in the following order; DADS> DAS> DATS> S_8. They were low because DAS, DADS and DATS, the main constituents in garlic oil, are volatile aliphatic disulfide and unstable. Therefore, during the storage and preparation, the loss of sulfur containing volatile might occur and leads to the reduction of sulfur compound in garlic oil. A large amount of DADS compared with DAS, DADS and DATS were consistent to previous results reported by Sheen et al. (1992) and O'Gara et al. (2000), DADS was found to be in higher concentration (33 %) compared to DATS (16.5%) and DAS (17.5%). Sheen et al. (1992) reported that garlic oil generally contains 40% DADS, 35% DATS, 10% DAS and other volatile compounds. From their results, DATS was found in higher concentration than DAS. The discrepancy in the concentration of sulfur compound in garlic oil was, perhaps, caused by of the loss of volatile components during storage, handing, and processing by spray drying. Table 4.5 showed the amount of DADS, DATS and S_8 in spray-dried garlic oil microcapsules prepared from garlic oil (0.2:1 oil to maltodextrin ratio) at various Tween®20 concentrations (0.6%, 0.8% and 1% w/w) in 20g/dL maltodextrin spray-dried at various temperatures (120, 160, 180 and 200°C) at a feed rate of 25 mL·min^{-1} and 3 bars. From Table 4.5, DAS, DADS, and DATS were reduced to virtually zero in all spray-dried microcapsules. Only S_8 was found in the samples, where the sample prepared from 0.6%, 0.8% and 1% w/w Tween® at an inlet air temperature of 120°C, 160 °C, 180°C and 200°C contained the highest amount of S_8 at 0.081 % w/w. The higher retention of S_8 in all samples compared to other organosulfur compounds was because of their cyclic molecular structure which yielded greater stability compared to the acyclic DAS, DADS and DATS (Block, 2009)

Table 4.5 Bioactive compounds of garlic oil microcapsules (0.2:1 oil to maltodextrin ratio) at various Tween®20 concentrations (0.6%, 0.8% and 1% w/w) in 20 g/dL maltodextrin spray-dried at various temperature (120, 160, 180 and 200°C) in a feed rate of 25 mL·min^{-1} and 3 bars.

Sample**	Inlet temp. (°C)	% Yield	Sulfur compounds (mg/g sample)			
			DADS*	DATS*	S_8*	Total (mg)
0.6% w/w Tween®20	120±5	22.5	0.00	0.00	0.45	0.45
	160±5	9.5	0.03	0.00	0.81	0.84
	180±5	9.5	0.05	0.00	0.63	0.68
	200±5	10.5	0.03	0.00	0.65	0.68
0.8% w/w Tween®20	120±5	25.0	0.00	0.00	0.36	0.36
	160±5	9.0	0.01	0.00	0.26	0.26
	180±5	7.0	0.02	0.01	0.21	0.24
	200±5	9.0	0.00	0.00	0.26	0.31
1% w/w Tween®20	120±5	24.5	0.00	0.00	0.33	0.33
	160±5	9.0	0.00	0.00	0.21	0.30
	180±5	10.5	0.00	0.00	0.28	0.28
	200±5	9.0	0.00	0.00	0.13	0.13

* DADS = Diallyl disulfides, DATS = Diallyl trisulfides, S_8 = Cyclic sulfurs

** Tween®20 in 20 g/dL maltodextrin solution with 0.2:1 garlic oil to maltodextrin

The reduction of sulfur compounds may directly result from the low retention of oil in microcapsule samples due to the highly volatile nature of the compounds. Re' (1998) stated that the exorbitant drying temperature affects the crust formation of microcapsules leading to the loss of volatile materials. The microcapsules spray-dried at an inlet air temperature of 180°C for the emulsion containing 0.6% w/w Tween®20 showed the higher DADS concentration than other samples. The DADS is an indicator for antimicrobial ability of garlic oil. Therefore, the sample was selected for further studies.

4.4 Antimicrobial ability

The garlic oil emulsions and microcapsule samples were determined for their MIC by the agar well diffusion method against *S. aureus* ATCC 25923, *S.* Typhimurium ATCC 13311 and *E. coli* ATCC 25922. The inhibition zone diameter was measured and the result of antimicrobial assay was discussed.

4.4.1 Antimicrobial assay of garlic oil emulsion by agar well diffusion method

The samples were tested for their inhibition against *S. aureus* ATCC 25923, *S.* Typhimurium ATCC 13311 and *E. coli* ATCC 25922 by agar well diffusion technique. Inhibition zone of all samples are shown in Figure 4.13.

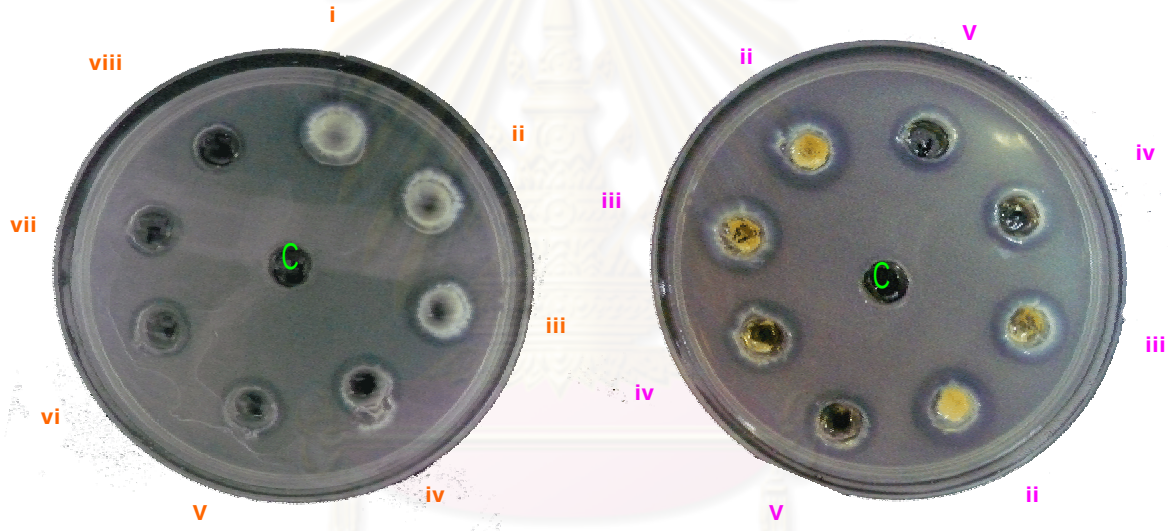


Figure 4.13 Inhibition zone of emulsion (A) and microcapsule (B) sample against pathogenic bacterium by agar well diffusion technique in 2-fold of 1 (i), 1/2 (ii), 1/4 (iii), 1/8 (iv), 1/16 (v), 1/32 (vi), 1/64 (vii) and 1/128 (viii).

Table 4.6 shows MIC and the width of inhibition zone of garlic oil emulsion containing various Tween®20 concentrations. MIC (0.5, 250, 125, 62.50, 31.25, 15.62, 7.81 μL/mL) against *S. aureus* ATCC 25923 was 31.25, 62.50 and 62.50 μL/mL, and the width of inhibition zone was 0.32, 0.30 and 0.40 cm, for the emulsion containing 0.6%, 0.8% and 1%w/w Tween®20 respectively. There was no inhibition against *S.* Typhimurium ATCC 13311 and *E. coli* ATCC 25922 for all emulsions in this study.

Table 4.6 MIC of garlic oil emulsions against *Staphylococcus aureus* ATCC 25923, *Samonella* Typhimurium ATCC 13311 and *Escherichia coli* ATCC 25922

Emulsifier (Tween®20) Concentration**	Tested Bacterium					
	S. aureus ATCC 25923		*Salmonella* Typhimurium ATCC 13311		*E. coli* ATCC 25922	
	MIC* (μL/mL)	Inhibition[a] zone (cm)	MIC* (μL/mL)	Inhibition[a] zone (cm)	MIC* (μL/mL)	Inhibition[a] zone (cm)
0.6%	31.25	0.32±0.08	NH	NH	NH	NH
0.8%	62.50	0.30±0.08	NH	NH	NH	NH
1%	62.50	0.40±0.06	NH	NH	NH	NH

* Each MIC determination was performed in triplicate per bacterial isolate
[a] Inhibition zone diameter (cm) did not include diameter of cock border (0.8 cm)
** Tween®20 in 20 g/dL maltodextrin solution with 0.2:1 garlic oil to maltodextrin
NH means "No inhibition"

The loss of antimicrobial activity of garlic oil emulsions against *Salmonella* Typhimurium ATCC 13311 and *Escherichia coli* ATCC 25922 may result from the low amount of garlic oil in the emulsion (0.04 g/mL). In addition, some of organosulfur compounds that possess antimicrobial activity might loss during preparation owing to their highly volatile properties. Yin and Chen (2002) reported the antimicrobial protection of DAS and DADS against the growth of *S. aureus*, *S.* Typhimurium and *E. coli* in ground beef. They reported that DAS and DADS could inhibit the growth of *S. aureus* at a greater extent compared to their inhibition against *Salmonella* Typhimurium and *E. coli*. Ten (10) μM of DADS outinhibited *S. aureus* compared to *Salmonella* Typhimurium and *E. coli* at 0.62 and 1.09 log CFU/g, respectively.

The prepared garlic oil emulsions contained 0.04 g of garlic oil per one gram of emulsion (0.2:1 oil to maltodextrin ratio in 20 g/dL Md solution). This means that one gram of emulsion composed of 0.052 g of DADS and 0.047 g of DADS. This was responsible for the fact that the emulsion possessed an antimicrobial activity against

S. aureus which is more susceptible to inhibition by the organo-sulfur compounds. Fujisawa et al. (2008) stated that the lower susceptibility to inhibition by the organo-sulfur compounds of gram negative *E. coli* compared with the gram positive *S. aureus* is due to the structure difference between bacterium of different gram classes, especially in their cell membrane. *E. coli* has 10 times higher content of lipid than *S. aureus* (Salton, 1964). Thereby, allicin or sulfur compounds may be trapped by this lipid layer and lose it potency to react with its major protein targets. This may explain why there was no inhibition activity of emulsions against *Salmonella* Typhimurium and *Escherichia coli*. For the effect of Tween®20 concentrations, the antimicrobial activity decreased with increasing Tween®20 concentration from 0.6% to 0.8% and 1% w/w. The bulky molecule of the surfactant (MW~1228 g/mol) might block or decrease the chance of contact between garlic oil droplets and target bacterium. The increase in emulsion viscosity due to surfactant might also decrease the diffusion ability of garlic oil droplets leading to a reduction in the antimicrobial activity of the emulsions.

4.4.2 Antimicrobial ability of garlic oil microcapsules

The microcapsules prepared from emulsions of garlic oil at various Tween®20 concentrations (0.6%, 0.8% and 1% w/w) in 20 g/dL maltodextrin spray-dried at various temperatures (120, 160, 180 and 200°C) at a feed rate of 25 mL·min^{-1} and 3 bars were tested for their antimicrobial ability against *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 13311 and *Escherichia coli* ATCC 25922 by agar well diffusion technique. Microcapsules from the emulsions that contain 0.6% and 0.8% w/w Tween®20 had an MIC of 0.1 g/mL. MIC of 0.2 g/mL was found in both microcapsules spray-dried at 200°C and 120°C of 0.6% and 0.8% w/w Tween®20 (Table 4.7). In consistent with the result presented in 4.4.1, the microcapsules had no inhibition against *Salmonella* Typhimurium and *Escherichia coli*. The increase in MIC was partly due to the reduction in the amount of garlic oil caused by heat evaporation and the reduction in the antimicrobial activity of the garlic oil due to heat the oil experienced during spray-drying process. The reduction in antimicrobial ability of garlic oil as a result of heat is discussed in section 4.4.3.

Table 4.7 MIC of garlic oil microcapsules against *Staphylococcus aureus* ATCC 25923, *Samonella* Typhimurium ATCC 13311 and *Escherichia coli* ATCC 25922

Emulsion**	Spray-dried Inlet temp. (°C)	Tested bacterium					
		S. aureus ATCC 25923		*Salmonella* Typhimurium		*E. coli* ATCC 25922	
		MIC* (g/mL)	Inhibition[a] Zone (cm)	MIC* (g/mL)	Inhibition[a] Zone (cm)	MIC* (g/mL)	Inhibition[a] Zone (cm)
0.6% w/w Tween®20	120±5	0.20	0.38±0.04	NH	NH	NH	NH
	160±5	0.10	0.45±0.07	NH	NH	NH	NH
	180±5	0.10	0.40±0.00	NH	NH	NH	NH
	200±5	0.10	0.30±0.14	NH	NH	NH	NH
0.8% w/w Tween®20.	120±5	0.20	0.40±0.00	NH	NH	NH	NH
	160±5	0.10	0.40±0.00	NH	NH	NH	NH
	180±5	0.10	0.30±0.00	NH	NH	NH	NH
	200±5	0.10	0.30±0.00	NH	NH	NH	NH
1% w/w Tween®20	120±5	0.10	0.35±0.07	NH	NH	NH	NH
	160±5	0.10	0.35±0.07	NH	NH	NH	NH
	180±5	0.10	0.35±0.07	NH	NH	NH	NH
	200±5	0.20	0.20±0.14	NH	NH	NH	NH

* Each MIC determination was performed in triplicate per bacterial isolate

[a] Inhibition zone diameter (cm) did not include diameter of cock border (0.8 cm)

** Tween®20 in 2o g/dL maltodextrin solution with 0.2:1 garlic oil to maltodextrin

NH means “Not inhibition”

4.4.3 Effect of temperature on microbial growth inhibition ability of garlic oil

Effect of temperature (70, 80 and 90°C) on the antimicrobial ability of garlic oil shown by the width inhibition zone of *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 13311 and *Escherichia coli* ATCC 25922 and *Escherichia coli* ATCC 25922 are presented in Figures 4.14 to 4.16. The garlic oil emulsion prepared by using an oil to maltodextrin ratio of 5:1 in 6% w/w Tween®20 in 20g/dL maltodextrin was heated at various temperatures (70°C, 80°C and 90°C) for 0.5 to 25 minutes. The results indicated that the inhibition of emulsion against all bacterium decreased with increasing time at 70, 80 and 90°C. The inhibition zone width observed for all emulsions heated at 90°C against *S. aureus* was the lowest compared to that at 70°C and 80°C. Taking the destruction of all bacteria as following first order kinetics, the reaction rate constants (k) of the destruction of *S. aureus* by the garlic oil emulsion heated at 70, 80 and 90°C were -0.0172, -0.0185 and -0.0098 min^{-1}, respectively (Table 4.8). The result obviously indicated that the antimicrobial ability of the emulsion against *S. aureus* reduced with heating temperatures. The destruction rate constant (k) of the emulsion heated at 90°C was approximately half of that at 70 and 80°C. However, there was no significant difference of k of *S. aureus* caused by the emulsion heated at 70, 80 and 90°C.

For the inhibition against *S.* Typhimurium and *E. coli*, the k caused by the emulsion heated at 70, 80 and 90°C was -0.0802, -0.0131 and -0.0904 min^{-1}, respectively for *S.* Typhimurium and -0.0197, -0.0329 and -0.0335 min^{-1}, respectively for *E. coli* (Table 4.8). The result did not show a decreasing trend for k value with increasing temperature. Similar to the result for *S. aureus*, statistical analysis showed that there was no significant difference of k of *S.* Typhimurium and *E. coli* caused by the emulsion heated at 70, 80 and 90°C.

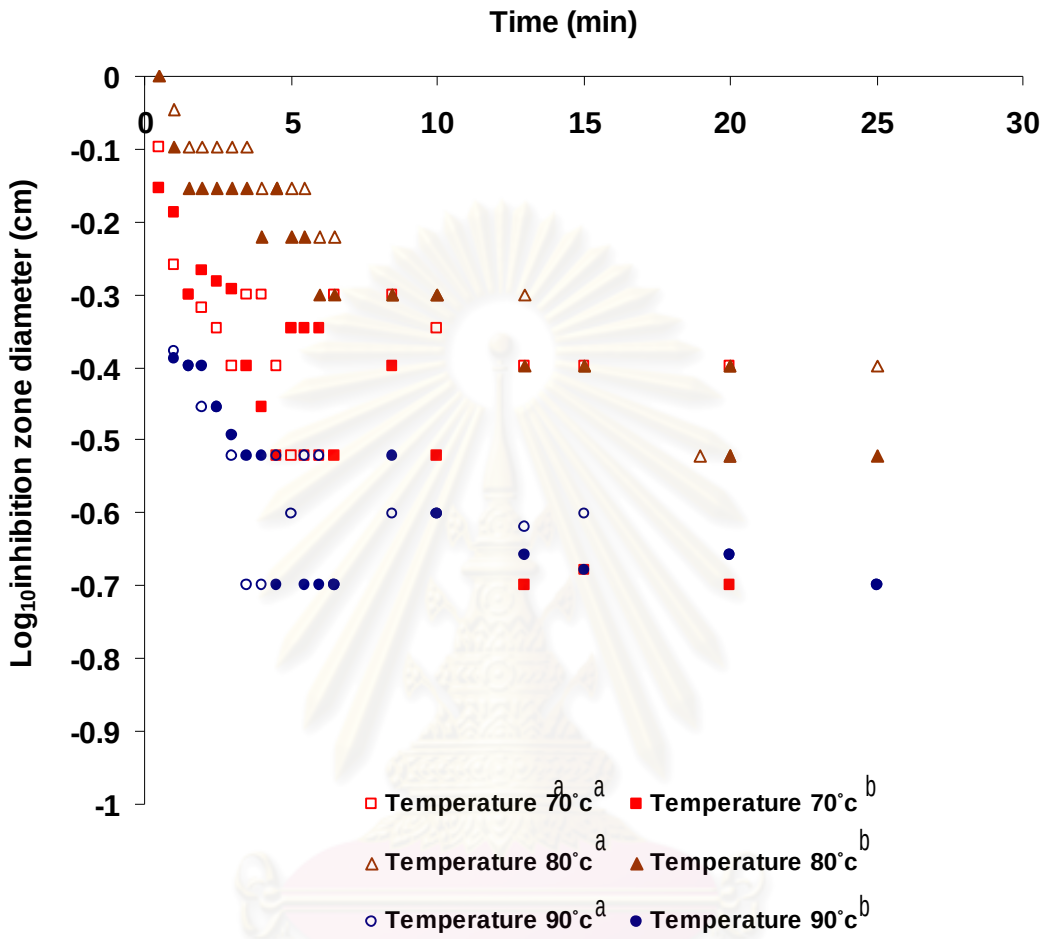


Figure 4.14 Inhibition ability against *Staphylococcus aureus* ATCC 25923 of garlic oil emulsion at various temperatures (70°C, 80°C and 90°C) for 0-25 minutes.
[a] First measurement, [b] Second measurement

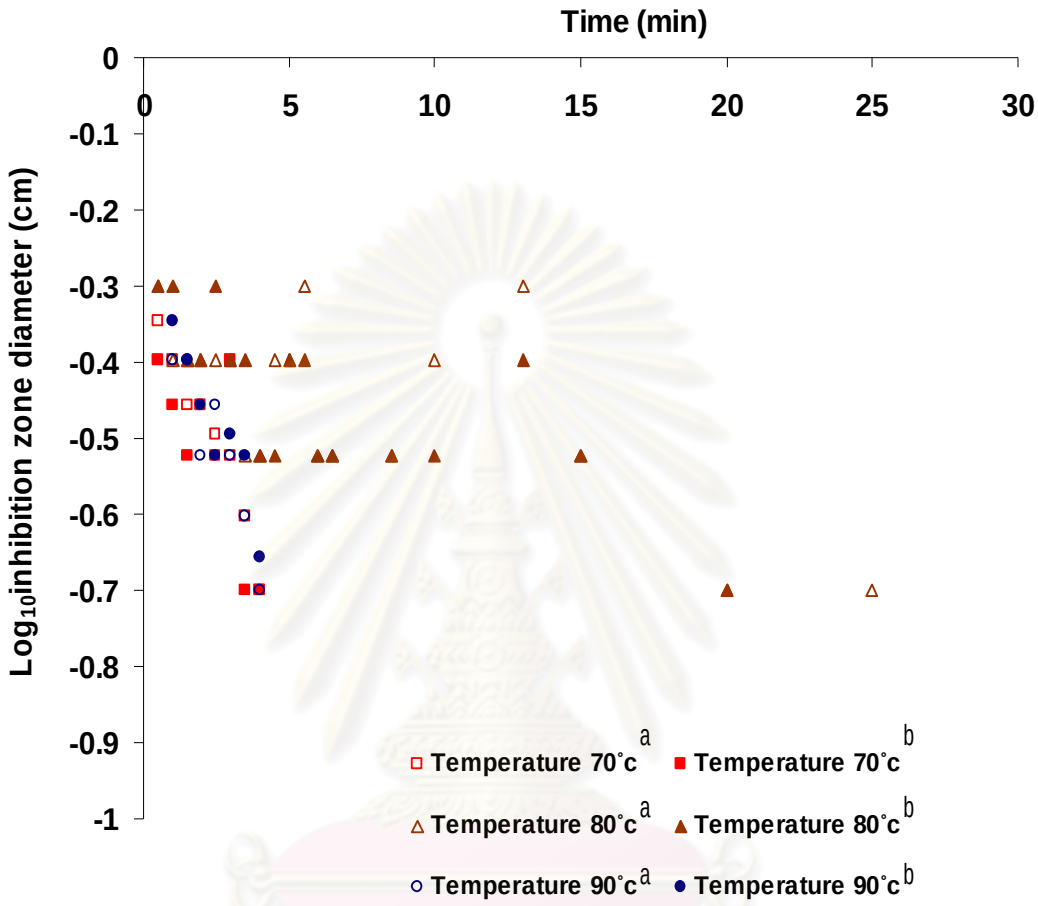


Figure 4.15 Inhibition ability against *Salmonella* Typhimurium ATCC 13311 of garlic oil emulsion at various temperatures (70°C, 80°C and 90°C) for 0-25 minutes.
[a] First measurement, [b] Second measurement

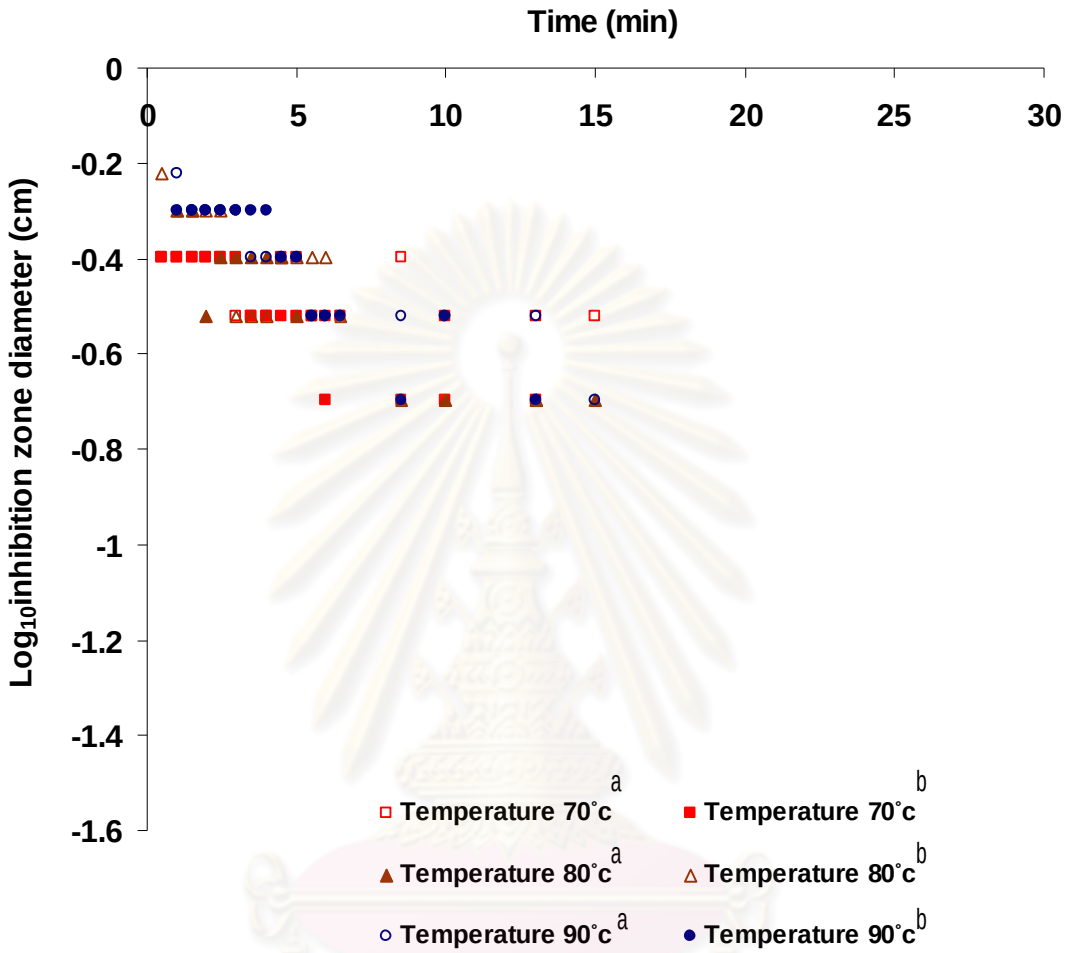


Figure 4.16 Inhibition ability against *Escherichia coli* ATCC 25922 of garlic oil emulsion at various temperatures (70°C, 80°C and 90°C) for 0-25 minutes.

[a] First measurement, [b] Second measurement

Table 4.8 kinetic parameter for thermal degradation of garlic oil in emulsion prepared by 5:1 oil to maltodextrin and 6% w/w Tween®20 in 20g/dL maltodextrin and heated at 70, 80 and 90°C

Test bacteria	Temperature(°C)	-k(min^{-1})
Staphylococcus aureus ATCC 25923	70	0.0172^{a} ±0.01
	80	0.0185^{a} ±0.00
	90	0.0098^{a} ±0.00
Salmonella Typhimurium ATCC 13311	70	0.0802^{b} ±0.01
	80	0.0131^{c} ±0.01
	90	0.0904^{b} ±0.00
Escherichia coli ATCC 25922	70	0.0197^{d} ±0.01
	80	0.0329^{d} ±0.01
	90	0.0335^{d} ±0.01

$^{a, b, c, d}$ Different letters in the same column denote significant difference ($p \leq 0.05$)

4.4.4 Evaluation of oil release in water from microencapsules

The release ability of garlic oil microcapsules prepared from the emulsion containing 0.2:1 garlic oil to maltodextrin and 0.6% Tween®20 in 20 g/dL maltodextrin and spray-dried at 180°C inlet air temperature was determined by using indirect method that involves measurement of the inhibition zone diameter against *Staphylococcus aureus* ATCC 25923. The width of inhibition zone of *S. aureus* is shown in Figure 4.17. The release of garlic oil from microcapsules in water was observed from 5 minutes onwards at 25°C. The release pattern of the microcapsules in water could, thus, be classified as burst release. The result shows that release of garlic oil was constant with increasing time.

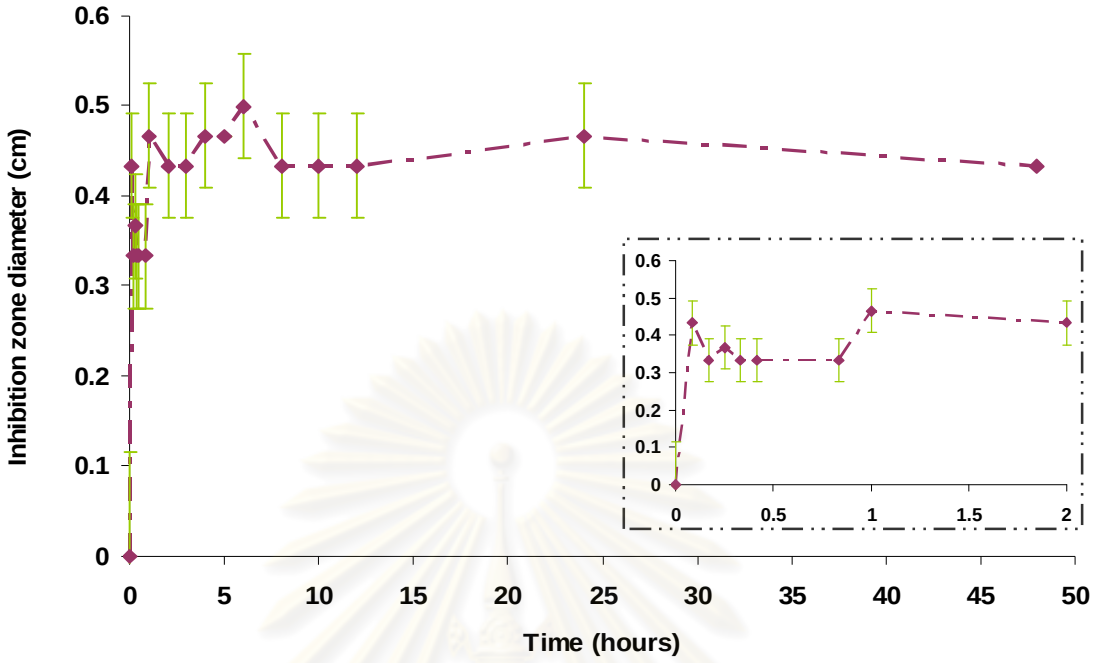


Figure 4.17 Diameter of inhibition zone against *Staphylococcus aureus* ATCC 25923 of garlic oil microcapsules at temperatures of 25°C for up to 48 hours.

4.5 Application of garlic oil microcapsules in salad dressing model

Garlic oil microcapsule (3.96 g) was added into 100 g of salad dressing model (pH 4.9). The salad dressing containing garlic oil microcapsules was stored at room temperature (~25°C) and was analyzed for total bacterial count during storage for 7 days.

4.5.1 Shelf life of salad dressing

Table 4.9 shows the total bacterial counts over 7 days of the salad dressing containing garlic oil microcapsules and the control salad dressing stored at 25°C for 7 days. The control dressing without antimicrobial microcapsules contained 0.9 log CFU/g and increased to 13.4 log CFU/g after storage at 25°C for 7 days, whereas the salad dressing containing garlic oil microcapsules contained 0.5 log CFU/g at the beginning and increased to 11.4 CFU/g at the 7th day of storage. This is apparent that the addition of garlic oil microcapsules could decrease the number of bacteria of

approximately 2 log CFU/g at the last day of storage. When handled according to Good Manufacturing Practice (GMP) conditions based on the General Principles of Food Hygiene (CAC, 2001a), aerobic plate count (APC) should be $\leq 10^5$ CFU/g. Total plate count of the salad dressing with garlic oil microcapsules and the control dressing was found to reach 10^5 CFU/g at day 3 and day 2, respectively. Thus, the addition of garlic oil microcapsules could extend the storage life of salad dressing model for 1 day at 25°C.

At day 0 of storage, the number of bacteria in salad dressing containing garlic oil microcapsules was lower than that in the control salad dressing. This could be due to the ability of microcapsules to reduce total bacteria from the beginning. When garlic oil was released from the microcapsules, it should react with target bacteria in the salad dressing. However, garlic oil consists of many sulfur compounds that may react with protein component in salad dressing, which caused the lower antibacterial ability.

Table 4.9 Total bacterial count in salad dressing containing garlic oil microcapsules and control salad dressing stored at 25°C for 1 to 7 days

Day	Total bacterial count (log_{10}CFU/g)	
	Control salad dressing	Salad dressing containing garlic oil microcapsules
0	$0.9^{ad}\pm0.07$	$0.5^{bd}\pm0.08$
1	$3.5^{ae}\pm0.19$	$2.6^{be}\pm0.08$
3	$6.5^{af}\pm0.12$	$4.9^{bf}\pm0.05$
5	$10.9^{ag}\pm0.13$	$7.3^{bg}\pm0.07$
7	$13.4^{ah}\pm0.01$	$11.4^{bh}\pm0.06$

[a, b] Different letters in the same row denote significant difference ($p\leq0.05$)

[d, e, f, g, h] Different letters in the same column denote significant difference ($p\leq0.05$)

4.5.2 Color of salad dressing

The color of salad dressing containing microcapsules was determined and compared with the control dressing. Table 4.10 shows that the color of the samples was significantly different ($p \leq 0.05$). The L*-value or lightness of salad dressing containing garlic oil microcapsules was higher than that of the control salad dressing. The +a*-value (redness) and +b*-value (yellowness) increased and decreased, respectively, when adding garlic oil microcapsules. The salad dressing containing garlic oil microcapsules appear to be more red and yellow. This was due to effect of release garlic oil droplet from microcapsules in the salad dressing and the natural color; dark brown, of garlic oil. The higher quantity of oil resulted in an increase in L*-value by increasing in dispersion and reflection of light (Chantrapornchai et al., 1999).

Table 4.10 Color of the control salad dressing and the salad dressing containing garlic oil microcapsules.

Sample	Color parameters		
	L*	a*	b*
Control salad dressing	67.64±0.01	-3.06±0.01	21.22±0.01
Salad dressing adding garlic oil microcapsules	68.67±0.06	-2.60±0.03	19.61±0.11

4.5.3 Sensory quality of salad dressing containing garlic oil microcapsules

The sensory assessments of salad dressing containing microcapsules and the control salad dressing were made. The assessors were asked to evaluate the samples for their color, flavor, odor, texture and overall acceptability by using descriptive analysis with scoring method. Figure 4.18 shows the sensory score for color, odor, texture (smoothness and viscosity) and the acceptability of salad dressing at day 1, 3 and 5 of storage at 25°C. Mean rating scores for color of control salad dressing and salad dressing containing garlic oil microcapsules at day 1, 3 and 5 of storage were 1.33, 2.58, 3.28 and 4.40, respectively, where 1 means light yellow and 7 means very dark

brown. Mean odor score for control salad dressing was 1.57, for salad dressing containing microcapsules at day 1, 3 and 5, the odor score were 6.37, 6.65 and 6.53, respectively, where 1 means odorless and 7 means extremely strong garlic odor. In comparison dressing with microcapsules was scored significantly higher for odor ($p \leq 0.05$) which may be caused the strong odor of garlic oil that has many volatile sulfur compounds. The scores for odor of salad dressing containing microcapsules at day 1, 3 and 5 were not significantly different ($p \leq 0.05$). Mean sensory score for smoothness of the control salad dressing and the salad dressing containing garlic oil microcapsules at day 1, 3 and 5 of storage were 4.63, 3.38, 3.28 and 4.40, respectively, where 1 means extremely smooth and 7 means not smooth. Although the salad dressing containing microcapsules at day 1 and 3 of storage was rated a lower score (more smooth) in comparison to the scores of control dressing, no significant difference ($p > 0.05$) was observed between the smoothness of the dressing at day 5 of storage and the control dressing. During aerobic storage of the salad dressing containing microcapsules for 5 days, high level of bacterial growth (~10^7CFU/g) could produce substances that resulted in denaturation of protein contained in salad dressing resulting from bacterial enzyme. For this reason, the smoothness of the dressing was lower. Mean score given by assessors for the viscosity of the dressing were 4.53, 5.75, 5.52 and 5.38 for control and salad dressing containing microcapsules at day 1, 3 and 5 of storage, respectively, where 1 means not viscous and 7 means extremely viscous. The salad dressing containing microcapsules received higher scores for viscosity when compared with the control dressing. This was due to the dissolution of maltodextrin from the microcapsules in the salad dressing. Mean acceptability score of the salad dressing containing microcapsules at day 1, 3 and 5 of storage and the control dressing were 2.50, 2.32, 2.02, and 5.17, respectively, where 1 means not acceptability and 7 means extremely acceptability. The salad dressing containing microcapsules was rated a much lower score compared to the acceptability scores of the control dressing ($p \leq 0.05$). This was mainly due to the strong garlic odor from the antimicrobial microcapsules added in the salad dressing.

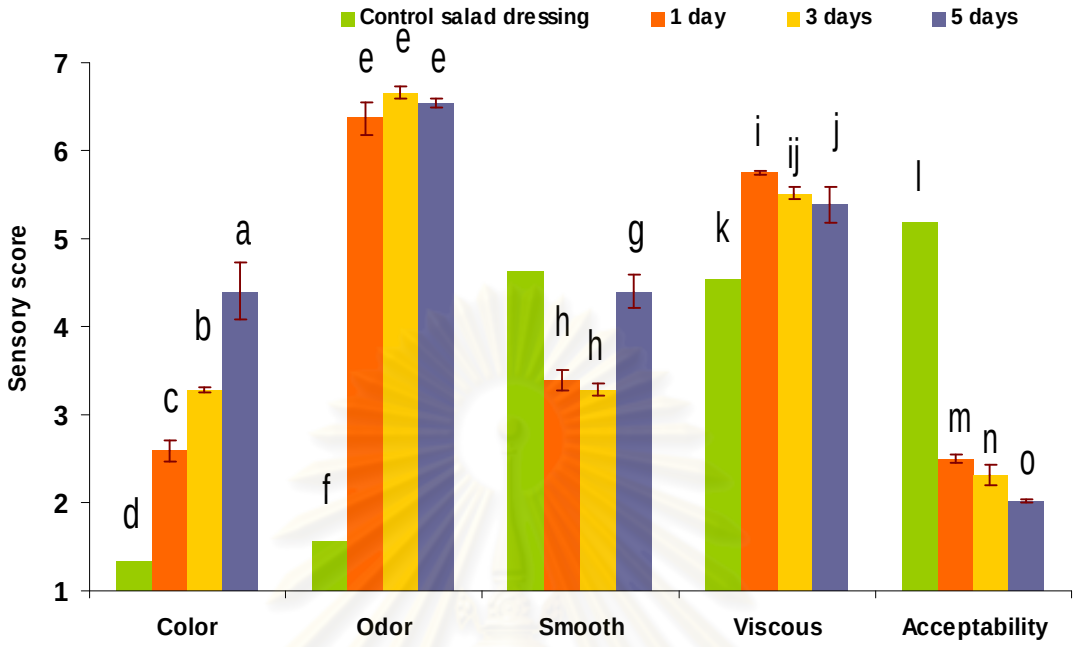


Figure 4.18 Sensory score for the salad dressing containing garlic oil microcapsules compared with the control salad dressing at day 1, 3 and 5 of storage at 25°C. a, b, c, d, e, f, g, h, I, j, k, l, m, n, o Different letters in the bar of the same group denote significant difference ($p \leq 0.05$)

CHAPTER V
CONCLUSIONS

The critical micelle concentration (CMC) of Tween®20 in 20 g/dL was 0.49%w/w for both Tween®20 and 80. Owing to the better solubility, Tween®20 was selected to prepare an emulsion in various Tween®20 concentrations (0.6%, 0.8% and 1% w/w). The emulsions were prepared from 0.6%, 0.8% and 1% w/w Tween®20 in 20 gl/dL maltodextrin solutions using oil to maltodextrin ratios of 0.1:1, 0.15:1 and 0.2:1 at 13,000, 19,000 and 24,000 rpm of rotational speed for 5 and 10 minutes, which provided the good stability at 25°C for 48 hours. The average droplet size of emulsions at 4°C for 24 hours ranged from 0.216 to 0.81 μm that the oil droplet size in an emulsion could be reduced by increasing the amount the rotational speed and the length of time to homogenize the sample. The containing of maltodextrin in an emulsion did not affect the oil droplet size significantly ($p>0.05$). After initial feed emulsion was spray-dried at various inlet air temperatures (120, 160, 180 and 200°C) at a feed rate of 25 mL/min and 3 bars, the microcapsules were produced. The particles appeared to be spherical and had smooth surface with small holes dispersing on their walls and large void in the center. Only particles prepared at an inlet air temperature of 120°C had rough or dented external surfaces that were a result of low inlet temperature. The average dried particle size D[3,2] ranged from 9 to 13 μm. The higher inlet air temperature decreased the moisture content and the water activity of microcapsules. Higher bulk density was observed for microparticles dried at 120°C. The solubility of all microcapsules was not significant difference ($p>0.05$). The microcapsules prepared from 0.6% w/w Tween®20 in 20 g/dL maltodextrin and 0.2:1 oil to maltodextrin spray-dried at 180°C possessed the highest oil retention and amount of diallyl sulfide compounds. Thus this was chosen for further studies on their release properties and application in salad dressing. The Minimum Inhibitory Concentration (MIC) against *Stapphyloccus aureus* ATCC 25923 of the selected emulsion and microcapsules was 31.25 μL/mL and 0.1 g/mL, respectively. The salad dressing containing garlic oil microcapsules (3.962×10^{-2} g/g) helped retard the spoilage of dressing by 1 day for storage at 25°C. The adding of microcapsules caused an increase in the light (L*) and redness (a*) but decreasing the yellowness (b*)

of salad dressing. This result was in accordant with the sensory assessment of the salad dressing containing garlic oil microcapsules. The color also tended to be more dark yellow during storage at 25°C for 5 days. The strong garlic oil odor might be the main reason for low acceptability score for the salad dressing containing garlic oil micrpcapsules product.

Suggestion

Garlic oil microcapsules could inhibit *Staphylococcus aureus* but not *Escherichia coli* and *Salmonella* Typhimurium because garlic oil is considered as effective at controlling a wide range of gram-positive bacteria which possess higher susceptibility to organo-sulfur compounds in the garlic oil. Garlic oil can possibly be active against gram-negative bacteria when it is applied at a higher concentration. Therefore, for future experiment, antimicrobial volatile oil microcapsules should be produced from more suitable polymer for spray drying which may combine various polymeric materials to protect and hold the highest amount of bioactive volatile oil in the microcapsules during spray drying at high temperature and during storage.

REFERENCES

Thai

นวลจิรา อนุสสรนิติสาร. 2527. เภสัชกรรมเทคโนโลยีของยาน้ำกระจายตัวและสารกึ่งแข็ง. คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล. กรุงเทพมหานคร.

ปรียา อาตมียะนันท์. 2525. ไมโครเอนแคปซูเลชัน. คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. กรุงเทพมหานคร.

สุกิจ นววงศ์. 2005. คู่มือวัตถุเจือปนอาหาร. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร: หจก. เอมี่ เทรดดิ้ง.

สุชาดา ประเสริฐวิทยาการ. 2527. อนุภาคศาสตร์. คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. กรุงเทพมหานคร.

วราภรณ์ สุวกูล. 2532. ปรากฏการณ์พื้นผิวและระหว่างพื้นผิว. คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. กรุงเทพมหานคร.

วราภรณ์ สุวกูล. 2527. อิมัลชัน. คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. กรุงเทพมหานคร.

English

Adetumbi, M., Javor, G.T., Lau, B.H.S. 1986. *Allium sativum* inhibits lipid synthesis by *Candida albicans*. Antimicrobial agents Chemotherapy. 30: 499-501

Ahmad, J. 1996. Garlic-a panacea for health and good taste. Nutrition and Food Science. 5: 32-35

Anker, M.H., Reineccius, G.A. 1988. Encapsulated Orange Oil: Influence of Spray-dryer Air Temperature on Retention and Shelf Life. In: Flavor Encapsulation, ASC symposium Series 370, American Chemical Society: Washingto D.C. 78-86.

Ankri, S., Miron, T., Rabinikov, A., Wilcheck, M., Marelman, D. 1997. Allicin from garlic strongly inhibits cysteine proteine proteases and cytopathic effects of *Entamoeba histolytica*. Antimicrobial agents Chemotherapy. 41: 2286-8

Ankri, S., and Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. Microbes and Infection. 2: 125-129.

Arshady, R. 1992. Naming microcapsules. Journal of Microencapsulation. 9: 187-190.

Association of Official Analytical Chemists (AOAC). 1990. Official methods of analysis. 15thed. Verginia: Association of official Agricultural Chemists Inc.

Association of Official Analytical Chemists (AOAC). 1995. Official methods of analysis. 16thed. Verginia: Association of official Agricultural Chemists International.

Aveyard, R., Binks, B.P. and Fletcher. 1990. The Structure, Dynamics ND Equilibrium Properties of Colloidal SystemsNATO ASI Ser. 324

Beristain, C.I., Garcia, H.S., and Vernon-Carter, E.J. 2001. Spray-dried Encapsulation of Cardamom (*Elettaria cardamom*) Essential Oil with Mesquite (*Prosopis juliflora*) Gum. Lebensmittel-Wissenschaft-Technologie. 34: 398-401.

Bhandari, B.R., Dumoulin, H.M.J., Richard, H.M.J., Noleau, I., and Lobert, A. M. 1992. Flavor encapsulation by spray drying: application to citral and linalyl acetate. Journal of Food Science. 57: 217-221.

Block, E., Ahmad, S., Catalfamo, J. L., Jain, M. K., and Apitz, C. R. 1986. Antithrombotic organosulfur compounds from garlic: structural, mechanistic, and synthetic studies. Journal of the American Chemical Society. 108: 7045-7055.

Block, E., 2009. Garlic and Other Alliums, The Lore and The science. 454

Brenner , R.R. 1981. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Progess in Lipid Reserch. 20: 41–47

Broadhead, J., Rouan, S.K.E., Hau, I., Rhodes, C.T. 1994. The effect of process an formulation variables on properties of spray-dried beta-galactosidase. Journal of Pharmacy and Pharmacology. 46: 458-467.

Bungenberg de Jong, H.G., Kaas, R. 1931. Biochem. Z. 232: 338- 345.

Cavallito, C.J., Bailey, H.J. 1944. Allicin, the antibacterial principle of Allium sativum L. Isolation, physical properties and antibacterial action. Journal of the American Chemical Society. 66: 1950-1.

Chandrakumer, M. 1995. From outbreak to prosecution. International Food Hygiene. 5, 27, 29.

Chantrapornchai, Clydesdale, W., McClements, D.J., 1999. Theoretical and Experimental Study of Spectral Reflectance and Color of Concentrated Oil-in-water Emulsions. Journal of Food Science. 218: 324-330.

Chung, J.G., Chen, G.W., Wu, L.T., Chang, H.L., Lin, J.G., Yeh, C.C., Wang, T.F. 1998. Effects of garlic compounds diallyl sulfide and diallyl disulfide on arylamine n-acetyltransferase activity in strains of *Helicobacter pylori* from peptic ulcer patients. American Journal of Chinese Medicine. 26: 353-364.

Conte, U., Conti, B., Guinchedi, P., Maggi, L. 1994. Spray dried polylactide microsphere preparation : influence of the technological preparation. Drug Development and Industrial Pharmacy. 20(3): 235-258.

Dickinson,E. 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloids, 17: 25-39.

Dickinson, E. 2006. Structure formation incasein-based gels, foams and emulsions. Colloids Surface and Physicochemical Engineering. 288-311.

Dokic, P., Jakovljevic, and Dokic-Baucal, Lj. 1997. Molecular characteristics of maltodextrins and rheological behaviour of diluted and concentrated solutions. Colloids and Surfaces. 141: 435-440.

Dziek, J. D. 1998. Microencapsulation and encapsulated ingredients. Food Technology. 42 (4): 36-151.

Erikson, J. P., Jenskins, P. 1991. Comparative *Salmonella* spp. and Listeria monocytogenes inactivation rates in four commercial mayonnaise products. Journal of Food Protection. 54 (12): 913-916

Farnath, K. 1997. Antioxidative activity of sulfur-containing flavor compounds in garlic. Bioscience Biotechnology and Biochemistry. 61: 1482-1485.

Fenwick, G.R., Hanley, A.B. 1985. Genus *Allium* part 1. CRC-Critical Reviews in Food Science and Nutrition. 22: 199-271.

Focke, M., Feld, A., Lichtenthaler, H.K. 1990. Allicin, a naturally occurring antibiotic from garlic, specifically inhibits acetyl-CoA-synthetase. FEBS letters. 261: 106-108.

Fujisawa, H., Suma, K., Origuchi, K.,Kumagai, H.,Seki, T.,Ariga, T. 2008. Biological And chemical stability of garlic-derived allicin. Journal of Agricultural and Food Chemistry. 56: 4229-4235.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R. 2007. Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Reseach International. 40: 1107–1121.

Gibbs, B.F., Kermasha, S., Alli, I., Mulligan, C.N. 1999. Encapsulation in the food industry: A review. International Journal of Food Science and Nutrition. 50: 213-224.

Glass, K.A., Loeffelholz, J., Harried, M., Nelson,J.H. 1993. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise dressing. Food Research Institute Annual Meeting, May 13.

Green, B.K., Schleicher, L. 1955. Pressure sensitive record materials. US Patent. 2: 217, 507.

Griffin, W.C. 1951. Solid essential oil concentrate and process of preparing the same. US Patent. 2: 556, 410.

Gungor, E., Gokoglu, N. 2008. Determination of microbial contamination sources at a frankfurter sausage processing line. Turkish Journal of Veterinary and Animal Sciences. 34(1): 53-59.

Han, J., Lawson, L., Han, G., Han, P. 1995. A spectophotometric method for quantitative determination of allicin and total garlic thiosulfinates. Analytical Biochemistry. 225: 157-160.

Hogan, S.A., McNamee, B.F., O'Riordan, E.D., and O'Sullivan, M. 2001. Emulsion and microencapsulation properties of sodium caseinate/carbohydrate blends. International Dairy Journal. 11: 137-144.

International Commission on Microbiological Specifications of Foods (ICMSF). 2005. Oil- and fat-based foods. Microorganisms in Foods 6: Microbial Ecology of Food Commodities. 6: 480-521.

Jafari, S.M., Assadpoor, E., Bhandari, B., He, Y. 2008. Nano-particle encapsulation of Fish oil by spray drying. Food Research International. 41(2): 172-183.

Kim, M.Y., Choi, S.W. and Chung, S.K. 2000. Antioxidative flavonoids from the garlic (*Allium sativum* L.) shoot. Food Science and Biotechnology. 9: 199–203.

Krest, I. 2000. Cysteine sulfoxides and alliinase activity of some *Allium* species. Journal of Agricultural and Food Chemistry. 48: 3753-3760.

Krstonosic, V., Dokic, L. Dokic, P., Dapcevic, T. 2009. Effects of xanthan gum on physico chemical properties and stability of corn oil-in-water emulsions stabilized by polyoxyethylene (20) sorbitanmonooleate. Food Hydrocoilliod. 23: 2213-2218

Lawson, L.D. 1993. Bioactive orgarnosulfur compounds of garlic and garlic products: Role in reducing blood lipids. Human Medical Agents from plants. 306-330.

Lawson, L.D. 1996. The composition and chemistry of garlic cloves and processed garlic. In: Garlic: The Science and Therapeutic Application of *Allium Sativum* L. and Related Species. 37-107.

Li, Y., Xu, S.Y. 2007. Preparation of garlic powder with high allicin content by using combined microwave-vacuum and vacuum drying as well as microencapsulation. Journal of Food Engineering. 83 (1): 76–83.

Lock, J.L., Board, R.G. 1994. The fate of *Salmonella enteritidis* PT4 in deliberately infected commercial mayonnaise. Food Microbiology. 11: 499-504.

Lock, J.L., Board, R.G. 1995. The fate of *Salmonella enteritidis* PT4 in home-made mayonnaise prepared from artificially incubated eggs. Food Microbiology. 12: 181-186.

Lowson, L.D. 2000. Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. Nutrition and Cancer. 38: 245-254.

Liu, X.D., ,Atarashi, T., Furuta, T., Yoshii, H., Aishima, S., Ohka, M. 2001. Microencapsulation of emulsified hydrophobic flavours by spray drying. Drying Technology. 19: 1361-1374

Lund, B.M., Parker, B., Gould, G.W. 2000. The microbiological safety and quality of food. Aspen Publishers, Maryland. 1: 175–199.

Madene, A., Jacquot, M., Scher, J., Desobry, S. 2006. Flavour encapsulation and controlled release-a review. Journal of Food Science and Technology. 41:1-21.

Malgorzata, G., Jeroen, H., Bongaerts, H., Jason, R.S., , Granick, S. 2007. Friction and adsorption of aqueous polyoxyethylene (Tween) surfactants at hydrophobic surfaces. Colloid and Interface Science. 315: 662-670

Masters, K. 1979. Spray drying handbook (3rd. ed.). New York: John Wiley and Son.

Mathiowitz, E., Chickering, D., Lehr, C.M. 1999. Bioadhesive Drug Delivery Systems Fundamentals. Novel Approaches and Development. 1-34.

McClements, D.J. 2005. Food Emulsions: Principles, Practice and Techniques. 2nd Edition, CRC Press. Boca Raton, FL

Meyer, M., Oxhoj, P. 1964. En musetyfusepidemi. Medlemsblad Danske Dyrlaegeforening. 47: 810-809.

Minemoto, Y., Hakamata, K., Adachi, S., Matsuno, R. 2002. Oxidation of linoleic acid encapsulated with gum arabic or maltodextrin by spray-drying. Journal of Microencapsulation. 19 (2): 181-189.

Mitchell, E.O, Mahoney, M., Lynch, D., Ward, L.R., Rowe, B., Uttley, A., Rogers, T., Cunningham, D.G., Watson, R. 1989. Large outbreak of food poisoning caused by *Salmonella Typhimurium* de type 49 in mayonnaise. British Medical Journal. 298: 99-101.

Nagpurgar, A., Peschell, J., and Holub, B. J. 2000. Garlic Constituents and Disease Prevention. Herbs, Botanicals and Teas. 1-21.

Newton, J.M. 1996. Spray drying and its application in pharmaceuticals. Manufacturing Chemist and Aerosol News. 33(4): 33-36, 35.

O'Gara, E.A., Hill, D.J., Maslin, D.J. 2000. Activities of garlic oil, garlic powder and their diallyl constituents against *Helicobacter pylori*. Application Environmental Microbiology. 66: 2269-2273.

Olsen, A.G., Seltzer, E. 1945. Preparation of flavouring materials. US Patent. 2: 369, 847.

Persyn, J., Oxlay, J. 2008. Micro/Nano Encapsulation. 1-12.

Prasad, K., Laxdal, V.A., Yu, M., Raney, B.L. 1996. Evaluation of hydroxyl radical-scavenging property of garlic. Molecular and Cellular Biochemistry. 154: 55–63.

Rabinkov, A., Miron, T., Konstantinovski, L., Wilchek, M., Mirelman, D., Weiner, L. 1998. The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochimica et Biophysica Acta. 1379: 233–244.

Re, M.I. 1998. Microencapsulation by spray drying. Drying Technology. 16: 6, 1195-1236

Rees, L.P., Minney, S.F., Plummer, N.T., Slater, J.H., and Skyrme, D.A. 1993. A quantitative assessment of the antimicrobial activity of garlic (*Allium sativum*). World Journal of Microbiology and Biotechnology. 9: 303-307.

Risch, S.J., Reineccius, G.A. 1988. Spray-Dried Orange Oil: Effect of emulsion size on Flavor retention and Shelf Stability. In: Flavor encapsulation ASC symposium Series 370, American Chemical Society: Washingto D.C. 67-77

Risch, S.J., Reineccius, G.A. 1995. In: Encapsulation and controlled Release of Food Ingredients. ASC symposium Series 590, American Chemical Society: Washingto D.C.

Rocha, G., Moore, P., Canto, L.R., Amante, E.R. 2005. Cassava and corn starch in maltodextrin production. Quimica Nova. 28(4): 596-600

Rosenberg, M., Kopelman, I.J., and Talmon, Y. 1985. A scanning electron microscopy study of microencapsulation. Journal of Food Science. 50 (1): 139-144.

Ross, Z.M., O'Gara, E.A., Hill, D.J., Sleightholme, H.V., Maslin, D.J. 2001. Antimicrobial properties of garlic oil against human enteric bacterial: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Applied and Environmental Microbiology. 67 (1): 475-480.

Salton, M. J. R. 1964. The Bacterial Cell Wall. London: Elsevier. 58.

Shahidi, F., and Han, X. 1993. Encapsulation of food ingredients. Critical Reviews in Food Science and Nutrition. 33(6): 501-507.

Sheen., L.Y., Lin, S.Y., Tsai, S.J., 1992. Odor assessments for volatile compounds of garlic and ginger essential oils by sniffing method of gas chromatography. Journal of Chinese Agricutural Chemical Society. 30: 14-24.

Sheu, T.Y., Rosenberg, M. 1998. Microstructure of microcapsules consisting of whey proteins and carbohydrates. Journal Food Science. 63: 491–494

Siems, W. G., Scherat, T., Behrend, H., Brenke, R., Jakstadt, M., Condari, E., and Grune, T. 1996. Influence of *Allium sativum* line on oxidative stress status: A clinical investigation. In: Proceedings of the International Symposium on Natural Antioxidants: Molecular Mechanisms and Health Effect. pp. 188-195.

Silva, J., Carvahlo, A. S., Teixeira, P., Gibbs, P. A. 2002. Bacteriocin production by spray dried lactic acid bacteria. Letters in Applied Microbiology. 34(2), 77–81.

Siripongvutikorn, S., Thummaratwasik, P. and Huang, W.Y. 2005. Antimicrobial and antioxidation effects of Thai seasoning, Tom-Yum. LWT-Food Science and Technology. 38: 347-352.

Snyder, O.P. 1998. Assuring safety of egg yolk-based sauces and salad dressing. Hospitality Institute of Technology and Management. USA.

Soottitantawat, A., Yoshii, H.,, Furuta, T., Ohgawara, M., Forssell, P., Partanen, R., Poutanen, K., Linko, P. 2004. Effect of water activity on the release characteristics and oxidative stability of d-limonene encapsulated by spray drying. Journal of Agricultural and Food Chemistry. 52(5): 1269-1276

Soottitantawat, A., Bigeard, F., Yoshii, H., Furuta T., Ohgawara, M., Linko, P. 2005. Influence of emulsion and powder size on the stability of encapsulated D-limonene by spray drying. Innovative Food Science and Emerging Technologies. 6(1): 107-114.

Sun, M.K., Kubota, K., and Kobayashi, A. 1997. Antioxidative activity of sulfur-containing flavor compounds in garlic. Bioscience Biotechnology and Biochemistry. 61: 1482-1485.

Telzak, E.E., Budnick, L.D., Greenberg. M.S., Blum, S., Shayegani, M., Benson, C.E., Schultz, S. 1990. A nosocomial outbreak of *Salmonella enteritidis* infection due to the consumption of raw eggs. The New England Journal of Medicine. 323: 394-397

The International Programme on Chemical Safety (IPCS). 1993. International Chemical Safety Card -- Benzoic acid. Geneva, World Health Organization, International Programme on Chemical Safety (ICSC 0103).

Todd, R. D. 1970. Microencapsulation and flavor industry. The Flavor Industry. 1: 768.

Tsao, S.M., Yin, M.C. 2001. In-vitro antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oils. Journal of Medical Microbiology. 50: 646-649.

Turchiuli, C., Fuchs, M., Bohin, M., Cuvelier, M.E., Ordonnaud, C., Peyrat-Maillard, M.N., and Dumoulin, E. 2004. Oil encapsulation by spray drying and fluidized bed agglomeration. Innovative Food Science and Emerging Technologies. 6: 29-35.

Wan, L.S.C., Heng, P.W.S., chia, C.G.H. 1990. Influence of operational and formulation factors on spray dried microcapsules. JSPS-NUS Seminar on Recent developments in pharmaceutical technology. 190-206.

Weiss, J.; Coupland, J. N., McClements, D. J. 1996. Solubilization of hydrocarbon droplets suspended in a nonionic surfactant solution. Journal of Physical Chemistry. 100: 1066-1071.

Wills, E.D. 1956. Enzyme inhibition by allicin, the active principle of garlic. Biochemical Journal. 63: 514-520.

Yin, M.C., Cheng, W. S. 1998. Antioxidant activity of several *Allium* members. Journal of Agricultural and Food Chemistry. 46: 4097-4101.

Yin, M.C., Cheng, W. S. 2002. Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. Meat Science. 63: 23-28.

Yu, L.,Shi-ying, X. 2007. Preparation of Garlic Powder with High Allicin Content. Agricultural Science in China. 6(7): 890-898.

Zakarian, J.A., King, C.J., 1982. Volatiles Loss in the Nozzle Zone During Spray Drying of Emulsions. Industrial and Engineering Chemistry Process Design and Development. 27:107-113.

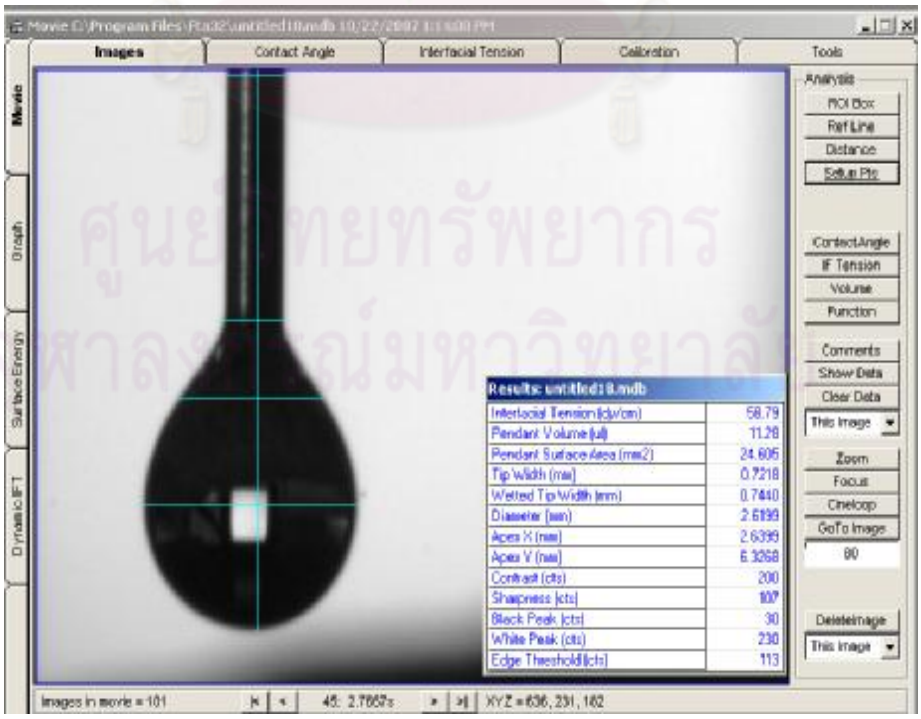
APPENDICES

Appendix A

Determination of physical and chemical properties

A1: Determination of surface tension by a goniometer

1. Place the sample in a 3 mL syringe (try to remove all air bubbles in the syringe).
2. Attach the needle, which has the inner and outer diameters of 0.483 and 0.711 mm, respectively, to the syringe.
3. Connect the syringe with the stand by using a syringe adapter and a gauge holder
4. Connect a camera at the video out socket.
5. Open the FTA program and set the pump rate to 12 μL/ second.
6. Run the machine and press IF tension to show the result.



A2: Bulk density (Beristain et al., 2001)

Method (Tapping method)

1. Weigh accurately 2 g of sample into a measuring cylinder and tap cylinder at altitude of 1 inch for every 1 minute for 15 times

2. Record the particles volume particles

Calculation

$$D = M / V$$

Where, D is bulk density ($g \cdot cm^{-1}$)

M is weight (g)

V is volume (mL)

A3: Moisture determination (AOAC, 1995) (number 925.10)

Instrument

1. Hot air oven (Model 600, Memmert, Gmiott Co. KG, Germany)

2. Desiccator

Methods

1. Weigh the aluminium dish, which has been previously dried in a hot air oven at 105°C until the weight of the dish is constant and then cool in a desiccator for an hour and weigh accurately the dish again.

2. Weigh accurately 2-3 g of sample into a moisture dish.

3. Place the dish in a hot air oven and dry at 105°C for 5 hours.

4. Remove the dish and cool the room temperature in a desiccator for an hour

Calculation

$$\text{Moisture (\%)} = ((W_1 - W_2) \times 100) / W_1$$

Where, W_1 is weight of the sample before drying (g)

W_2 is weight of the sample after drying (g)

A4: Water activity determination

Instrument

1. Water activity analyzer (AquaLab Series 3, Decagon Devices, Inc., USA)

Method

1. Weigh accurately 2-3 g of sample into a tray and close the cap for an hour.
2. Open the cap and place the tray in the water activity analyzer.
3. Measure and record the water activity value that is shown on the monitor.

A5: Solubility test (Modified from Jangchud and Chinnan (1999))

Instrument

1. Hot air oven (Model 600, Memmert, Gmiott Co. KG, Germany)
2. Dessicator

Method

1. Weigh Whatman No.1 filter paper and aluminium dish that has been previously dried in a hot air oven at 105^{o}C until the weight of the dish is constant. The filter paper and the dish were then cooled in a dessiccator for an hour and weighed. The drying was carried out until the weight is constant.
2. Weigh accurately 2 g of sample and dissolve in 20 mL distilled water and mix for an hour.
3. Filter the suspension through the pre-dried Whatman No.1 filter paper.
4. Place paper on a moisture dish and dry at 105^{o}C for 5 hours.
5. Remove the dish and leave it to cool to room temperature in a dessiccator for an hour before weighing.

Calculation

Solubility (%) = $(1-((W_1 - W_2)/ W_3)) \times 100$

Where, W_1 is weight of filter paper after drying (g)

W_2 is weight of filter paper before filtration (g)

W_3 is weight of the sample (g)

Appendix B

Microbial Determination

B1: Agar well diffusion technique (Modified from that of Parente et al., 1995 and Dawson et al., 2003)

1. Innoculate 100 μL of broth of test bacteria (10^6 CFU/mL) into a plate.

2. Add twenty (20) mL of the appropriate sterilized nutrient semi solid agar (Nutrient broth+1% agar that was held in solution at 42°C) into the plate and pour plate and cool to room temperature to allow agar solidification.

3. Makes test wells using a sterile 8 mm diameter cork borer.

4. Dispense test solutions into individual wells (100 μL per well).

5. Incubate plate overnight at 37°C in aerobic condition until growth of the test organism was observed.

6. Measure the diameter of inhibition zone (cm) using a vernier caliper.

B2: Determination of aerobic plate count (APC) by viable plate count method (Modified from that of Gungor and Gokoglu, 2008

Quantifying bacteria by spread plate in the following steps;

1. Add plate count agar medium (PCA) into a plate and cool to room temperature to allow agar solidification (the surface of solid medium have to be absolutely dried).

2. Take 0.1 mL of the diluted sample solution for each dilution sample into PCA plate and spread the diluted sample by a sterile glass spreader. The experiment was done in 3 replications and turn upside down the plate and incubate plate overnight at 37°C for 48 hours and count colonies (30 to 300 colonies) to calculate the colony forming unit per gram of sample (CFU/g).

Appendix C

Salad dressing

C1: Preparation of salad dressing model

Salad dressing on 1 unit of recipe;

Ingredients

1. Mustard	10.5	g
2. Egg yolk	170	g
3. Vegetable oil	250	g
4. Lime juice	25	g
5. Salt	5	g
6. Pepper	2	g
7. Sweetened condensed milk	360	g
8. Fresh milk	240	g

Method

1. Mix mustard, salt, pepper and lime together and set aside.

2. Whip egg yolk using high speed egg whisk and slowly add 1/3 part of oil, 1 teaspoon at a time.

3. Add the rest of oil alternately with the mixture in No.1.

4. Decrease the whipping speed of egg whisk and add both sweetened condensed milk and fresh milk. Mix all ingredients together.

C2: Evaluation sheet for sensory assessment

Sensory assessment of Salad dressing containing antimicrobial microcapsules

Name.....Date.....

Suggestion : Please consider and evaluate the samples for their appearance by marking √ into the blank that could be best explained the attribute.

Appearance	Sample code		
	R	.....	.....
1) Color -Very dark brown (7) -Dark brown (6) -Brown (5) -Light brown (4) -Very dark Yellow (3) -Dark Yellow (2) -Light Yellow (1)			
2) Odor -Extremely strong odor (7) -Very strong garlic odor (6) -Strong garlic odor (5) -Moderately strong garlic odor (4) - Mild garlic odor (3) -Very mild garlic odor (2) -Odorless (1)			
3.1) Smoothness (texture) -Extremely smooth (7) -Highly smooth (6) -Very smooth (5) -Smooth (4) -Rather smooth (3) -Quite smooth (2) -Not smooth (1)			

Appearance	Sample code		
	R	.....	.....
3.2) Viscosity (Texture) -Extremely viscous (7) -Highly viscous (6) -Rather viscous (5) -Moderately viscous (4) -Low viscosity (3) -Very low viscosity (2) -Not viscous (1)			
4) Acceptability -Not acceptable (1) -Quite acceptable (2) -Rather acceptable (3) -Acceptable (4) -Very acceptable (5) -Highly acceptable (6) -Extremely acceptable (7)			

Appendix D

Garlic oil emulsion appearance, stability, oil droplet size, bioactive compounds microbial assay and sensory assessment

Table D1 The appearance of garlic oil emulsion of 0.1:1 oil to maltodextrin (MD) in 0.6% Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD	rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution	Homogenous milky white solution
		24,000	5	Homogenous milky white solution	Homogenous milky white solution
			10	Homogenous milky white solution	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D2 The appearance of garlic oil emulsion of 0.15:1 oil to maltodextrin (MD) in 0.6% Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD	rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
		24,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D3 The appearance of garlic oil emulsion of 0.2:1 oil to maltodextrin (MD) in 0.6% Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD	rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
		24,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D4 The appearance of garlic oil emulsion of 0.1:1 oil to maltodextrin in (MD) 0.8% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD**	Rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
		24,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D5 The appearance of garlic oil emulsion of 0.15:1 oil to maltodextrin (MD) in 0.8% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD	Rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
		24,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D6 The appearance of garlic oil emulsion of 0.2:1 oil to maltodextrin (MD) in 0.8% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD	Rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
		24,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D7 The appearance of garlic oil emulsion of 0.1:1 oil to maltodextrin (MD) in 1% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD	Rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
		24,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D8 The appearance of garlic oil emulsion of 0.15:1 oil to maltodextrin (MD) in 1% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD	Rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
		24,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D10 The appearance of garlic oil emulsion of 0.2:1 oil to maltodextrin (MD) in 1% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored for 48 hours of storage at 25°C

Ratio		Homogenization condition		Emulsion appearance*	
				in 20g/dL maltodextrin continuous phase	In distilled water continuous phase
Oil	MD	Rpm	Time (min)		
0.1	1	13,000	5	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
			10	Homogenous milky white solution with precipitate in the bottom	Two separated layers; milky white solution on top and yellow solution in the bottom
		19,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
		24,000	5	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution
			10	Homogenous milky white solution with precipitate in the bottom	Homogenous milky white solution

* Experiments were done in 2 replications.

Table D11 Oil droplet size D[3,2] in garlic oil emulsion containing 0.1:1, 0.15 and 0.2:1 of oil to maltodextrin (MD) ratio in 0.6% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored at 4°C overnight.

Ratio		Operation of Homogenization		Oil droplet size D[3,2] in emulsion*	
				in 20g/dL maltodextrin continuous phase	In distilled water[ns] continuous phase
Oil	MD	rpm	Time (min)		
0.1	1	13,000	5	$0.223^{abcd}\pm0.030$	0.197 ± 0.031
			10	$0.209^{abcd}\pm0.002$	0.219 ± 0.019
		19,000	5	$0.147^{fgh}\pm0.008$	0.165 ± 0.013
			10	$0.147^{fgh}\pm0.008$	0.126 ± 0.012
		24,000	5	$0.134^{h}\pm0.001$	0.135 ± 0.008
			10	$0.153^{efgh}\pm0.030$	0.139 ± 0.028
0.15	1	13,000	5	$0.222^{abcd}\pm0.001$	0.177 ± 0.002
			10	$0.224^{abc}\pm0.027$	0.217 ± 0.011
		19,000	5	$0.190^{abcdefg}\pm0.021$	0.184 ± 0.001
			10	$0.184^{efgh}\pm0.004$	0.161 ± 0.018
		24,000	5	$0.173^{efgh}\pm0.021$	0.163 ± 0.002
			10	$0.176^{efgh}\pm0.008$	0.157 ± 0.012
1	1	13,000	5	$0.229^{ab}\pm0.024$	0.260 ± 0.036
			10	$0.239^{a}\pm0.032$	0.236 ± 0.044
		19,000	5	$0.141^{gh}\pm0.008$	0.216 ± 0.007
			10	$0.209^{abcd}\pm0.013$	0.193 ± 0.013
		24,000	5	$0.195^{abcdef}\pm0.045$	0.208 ± 0.022
			10	$0.200^{abcde}\pm0.019$	0.193 ± 0.016

* values from three measurements and two experiments.

[a,b,c,d,e,f] Different letters in the same column denote significant difference ($p\leq0.05$).

[ns] in the same row denote significant difference ($p\leq0.05$).

Table D12 Oil droplet size D[3,2] in garlic oil emulsion containing 0.1:1, 0.15 and 0.2:1 of oil to maltodextrin (MD) ratio in 0.8% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored at 4°C overnight.

Ratio		Operation of Homogenization		Oil droplet size D[3,2] in emulsion*	
				in 20g/dL maltodextrin continuous phase	In distilled water[ns] continuous phase
Oil	Malto	rpm	Time (min)		
0.1	1	13,000	5	0.227^{abc}±0.007	0.182±0.022
			10	0.242^{a}±0.062	0.202±0.013
		19,000	5	0.172^{def}±0.031	0.141±0.007
			10	0.134^{ef}±0.002	0.124±0.006
		24,000	5	0.183^{ef}±0.004	0.139±0.021
			10	0.126^{f}±0.008	0.213±0.003
0.15	1	13,000	5	0.237^{ab}±0.017	0.194±0.008
			10	0.200^{abcd}±0.039	0.207±0.018
		19,000	5	0.172^{def}±0.001	0.178±0.027
			10	0.162^{def}±0.001	0.151±0.003
		24,000	5	0.152^{def}±0.013	0.147±0.012
			10	0.153^{def}±0.001	0.154±0.026
1	1	13,000	5	0.237^{ab}±0.001	0.210±0.038
			10	0.233^{ab}±0.001	0.247±0.042
		19,000	5	0.198^{abcd}±0.018	0.201±0.001
			10	0.203^{bcd}±0.039	0.184±0.003
		24,000	5	0.186^{bcde}±0.016	0.188±0.009
			10	0.176^{cdef}±0.006	0.189±0.007

* values from three measurements and two experiments.

[a,b,c,d,e,f] Different letters in the same column denote significant difference ($p \leq 0.05$).

[ns] in the same row denote significant difference ($p \leq 0.05$).

Table D13 Oil droplet size D[3,2] in garlic oil emulsion containing 0.1:1, 0.15 and 0.2:1 of oil to maltodextrin (MD) ratio in 1% w/w Tween®20 in 20 g/dL maltodextrin solution and in distilled water stored at 4°C overnight.

Ratio		Operation of Homogenization		Oil droplet size D[3,2] in emulsion*	
				in 20g/dL maltodextrin continuous phase	In distilled water^{ns} continuous phase
Oil	Malto	rpm	Time (min)		
0.1	1	13,000	5	0.232^{abcde}±0.001	0.152±0.027
			10	0.201^{abcdef}±0.01	0.179±0.009
		19,000	5	0.147^{def} ±0.017	0.156±0.009
			10	0.129^{f} ±0.003	0.140±0.030
		24,000	5	0.130^{f}±0.013	0.166±0.005
			10	0.127^{f}±0.015	0.111±0.001
0.15	1	13,000	5	0.281^{a}±0.008	0.229±0.084
			10	0.237^{abcd}±0.057	0.197±0.025
		19,000	5	0.174^{cdef}±0.036	0.215±0.011
			10	0.154^{def}±0.008	0.132±0.013
		24,000	5	0.241^{abcd}±0.001	0.136±0.004
			10	0.140^{ef}±0.001	0.173±0.071
1	1	13,000	5	0.273^{ab}±0.018	0.222±0.033
			10	0.256^{abc}±0.008	0.249±0.023
		19,000	5	0.196^{abcdef}±0.004	0.187±0.001
			10	0.183^{bcdef}±0.009	0.153±0.006
		24,000	5	0.191^{abcdef}±0.039	0.162±0.019
			10	0.185^{abcdef}±0.031	0.142±0.004

* values from three measurements and two experiment

^{ns} in the same row denote significant difference ($p \leq 0.05$)

Table D14 Refractive index of distilled water, Tween®20, 20 g/dL maltodextrin solution and garlic oil.

Sample	Refractive index*
Distilled water	1.330±0.00
20 g/dL Maltodextrin solution	1.360±0.00
Tween®20	1.469±0.00
Garlic oil	1.5770±0.00

* values from three measurements

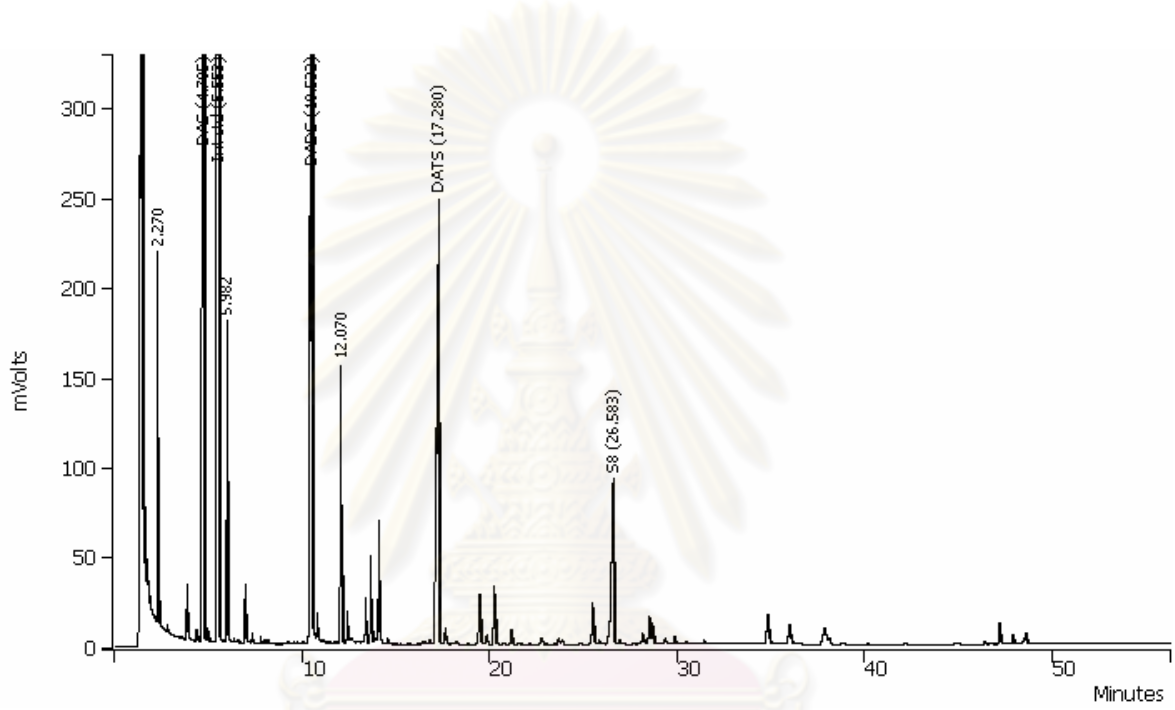
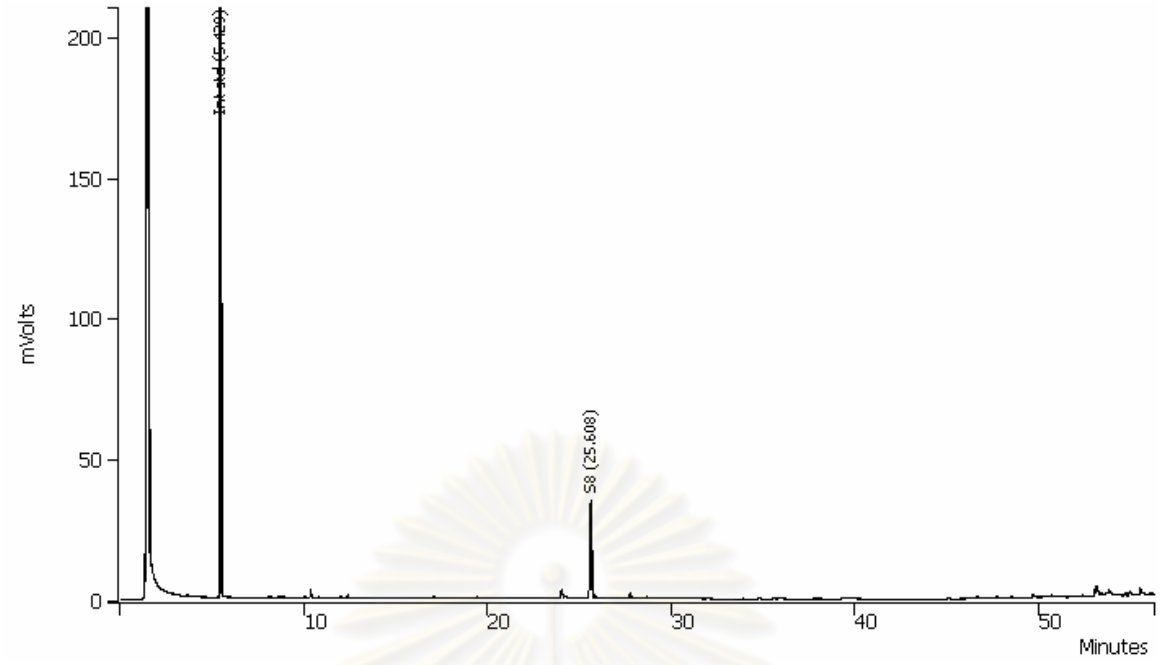
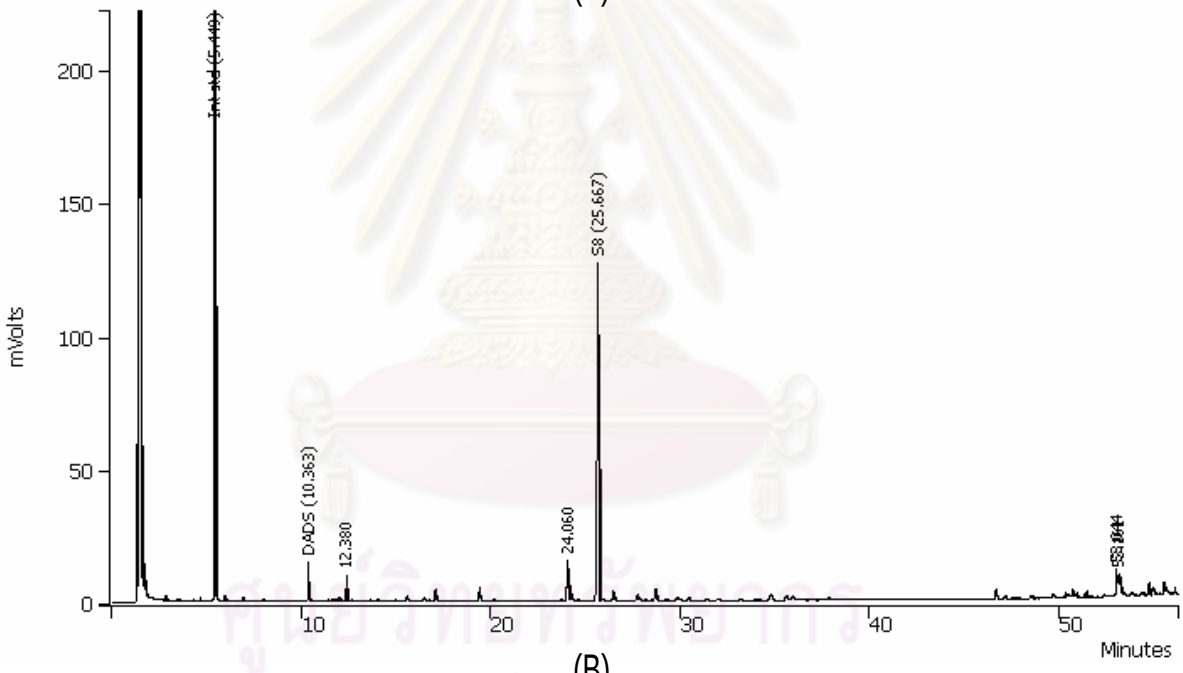


Figure D1 Typical chromatograms of garlic oil. The initial on the chromatograms indicates the compounds as below: Diallyl sulfides (DAS), Dialyll disulfides (DADS), Dialyll trisulfides and cyclic sulfurs (S_8).

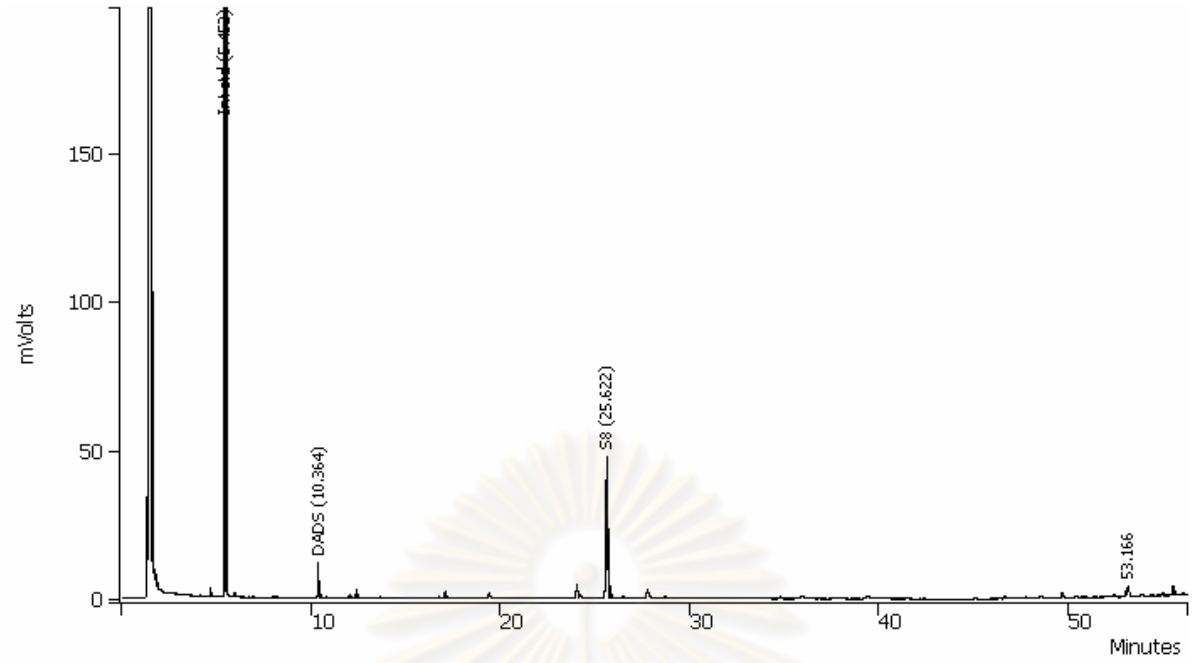


(A)

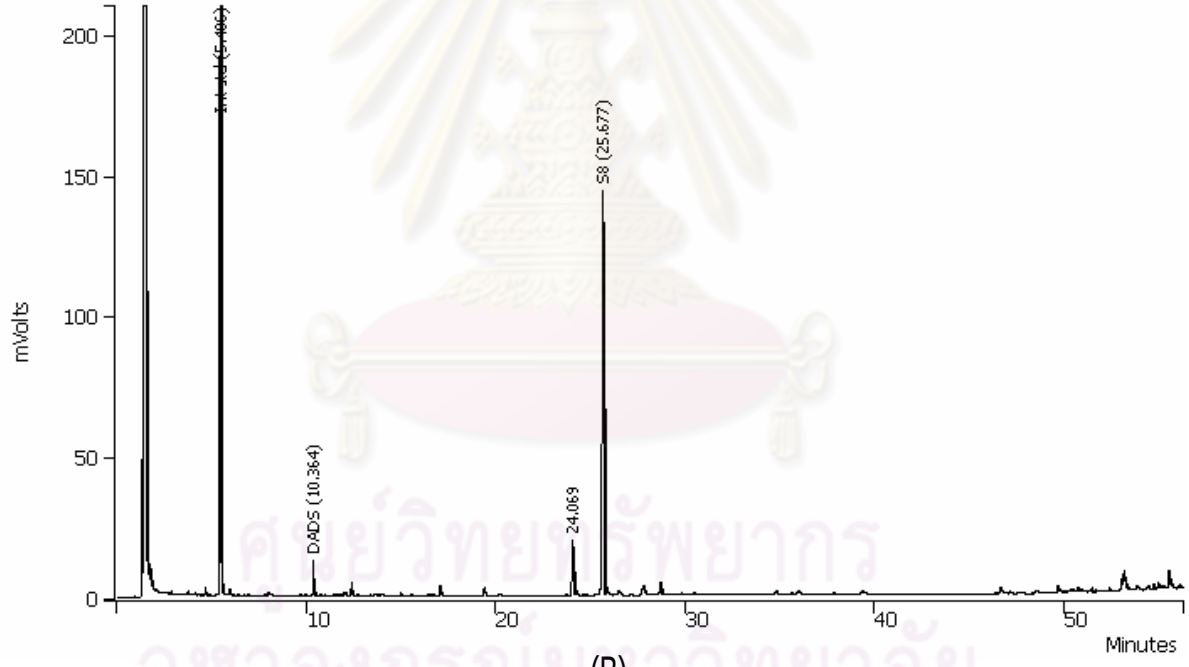


(B)

Figure D2 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 0.6% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 160±5°C for the 1st (A) and 2nd (B) experiments are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)

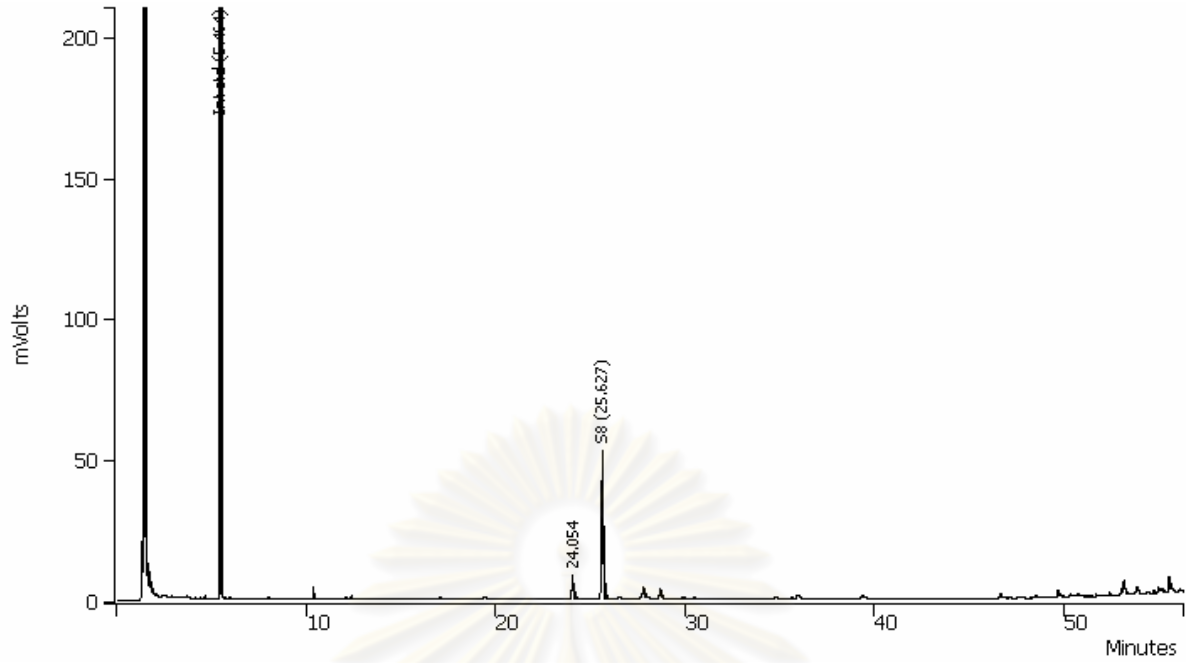


(A)

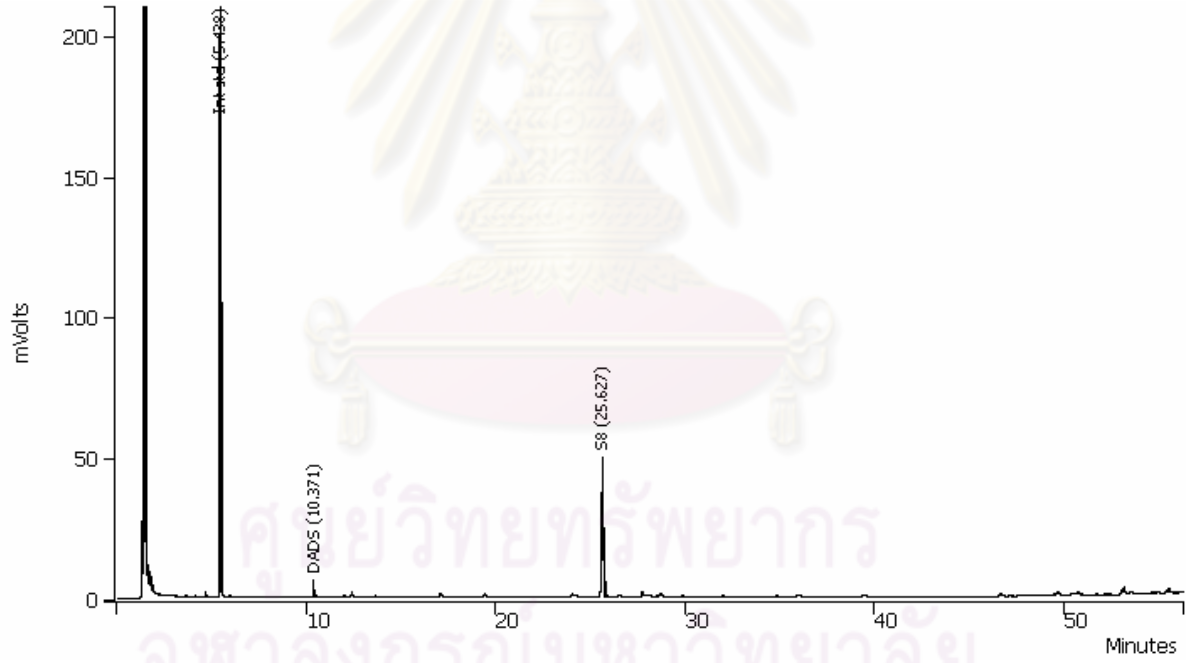


(B)

Figure D3 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 0.6% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 180±5°C for the 1st (A) and 2nd (B) experiments are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)

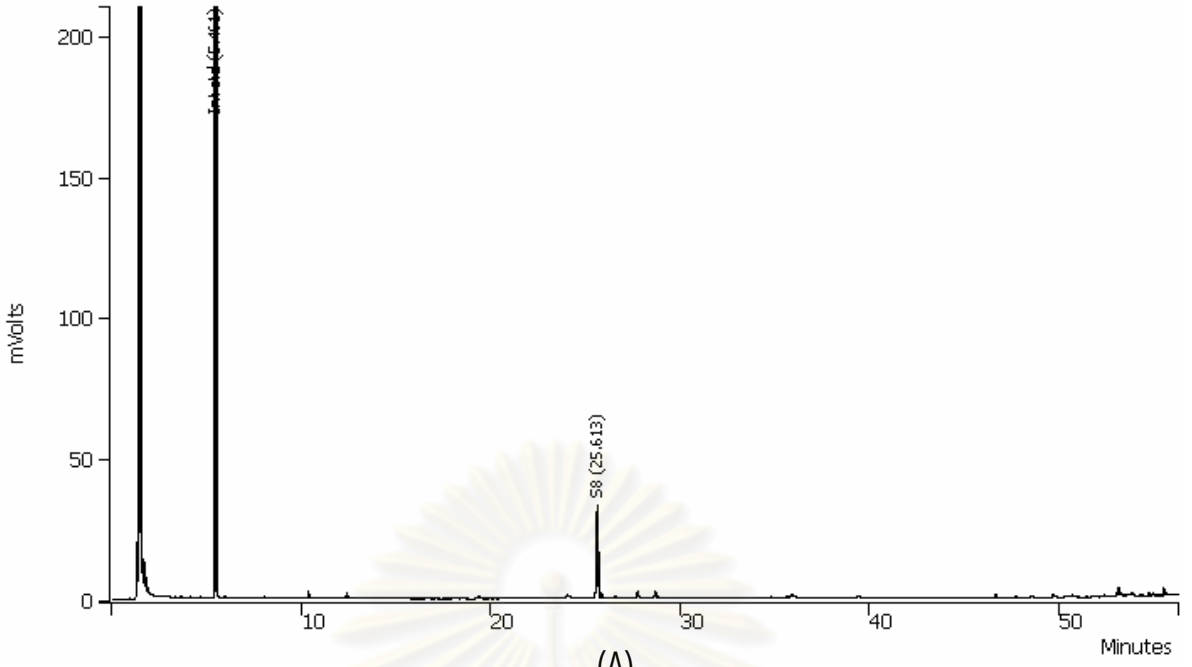


(A)

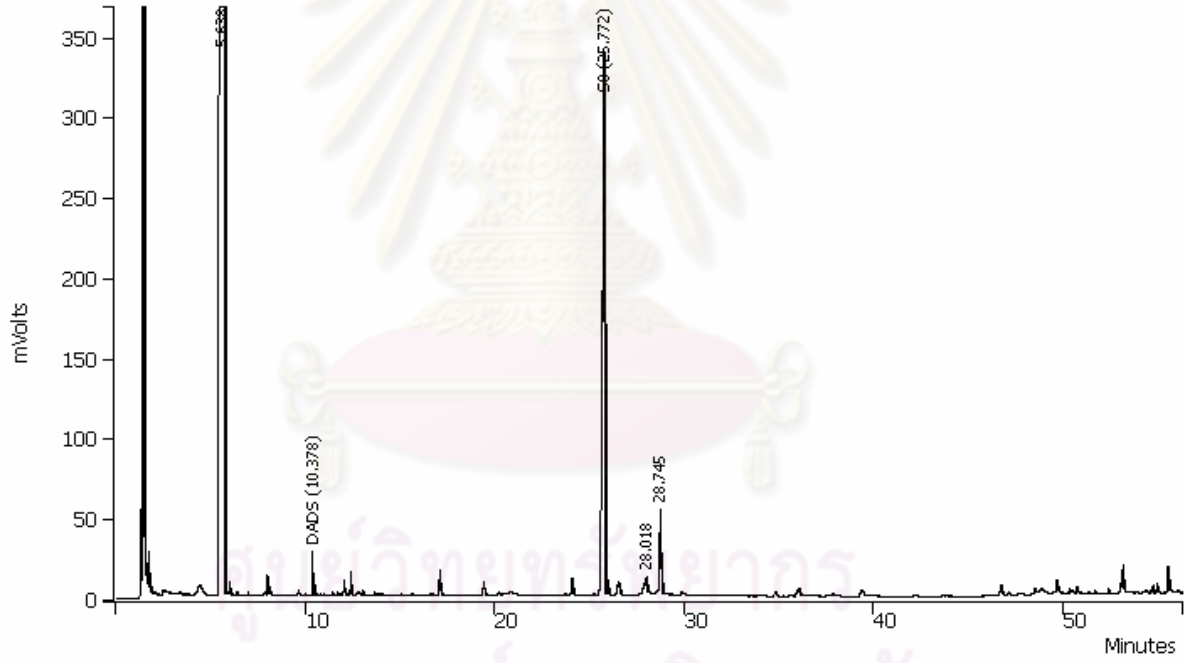


(B)

Figure D4 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 0.6% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 200±5°C for the 1st (A) and 2nd (B) experiments are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)

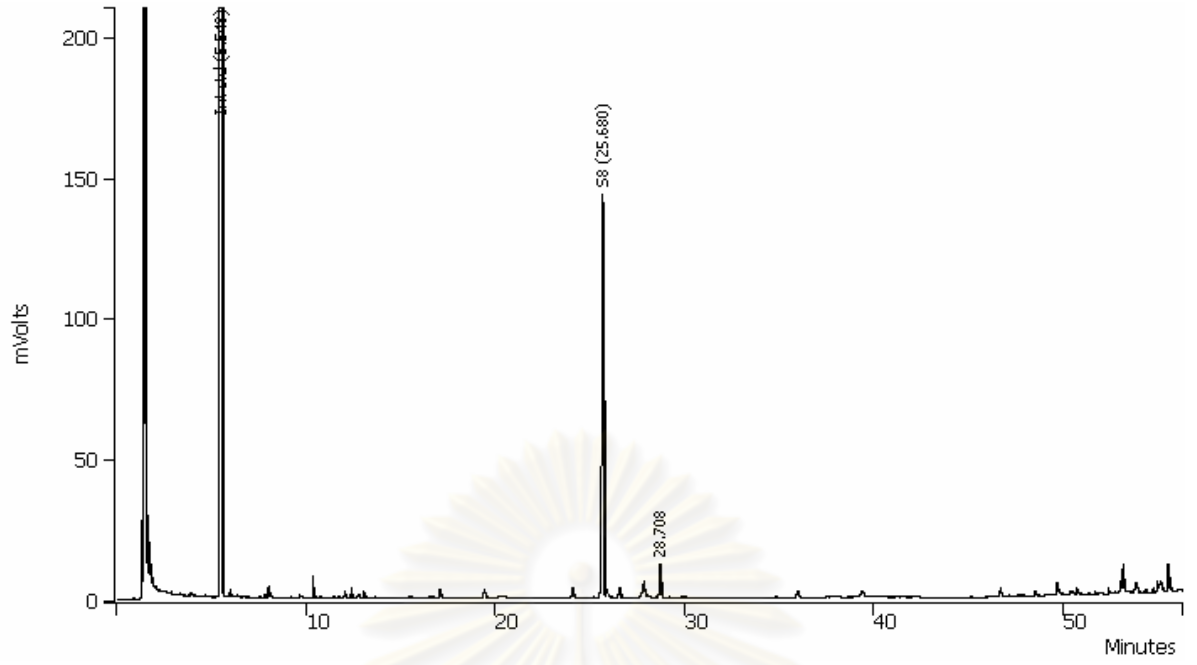


(A)

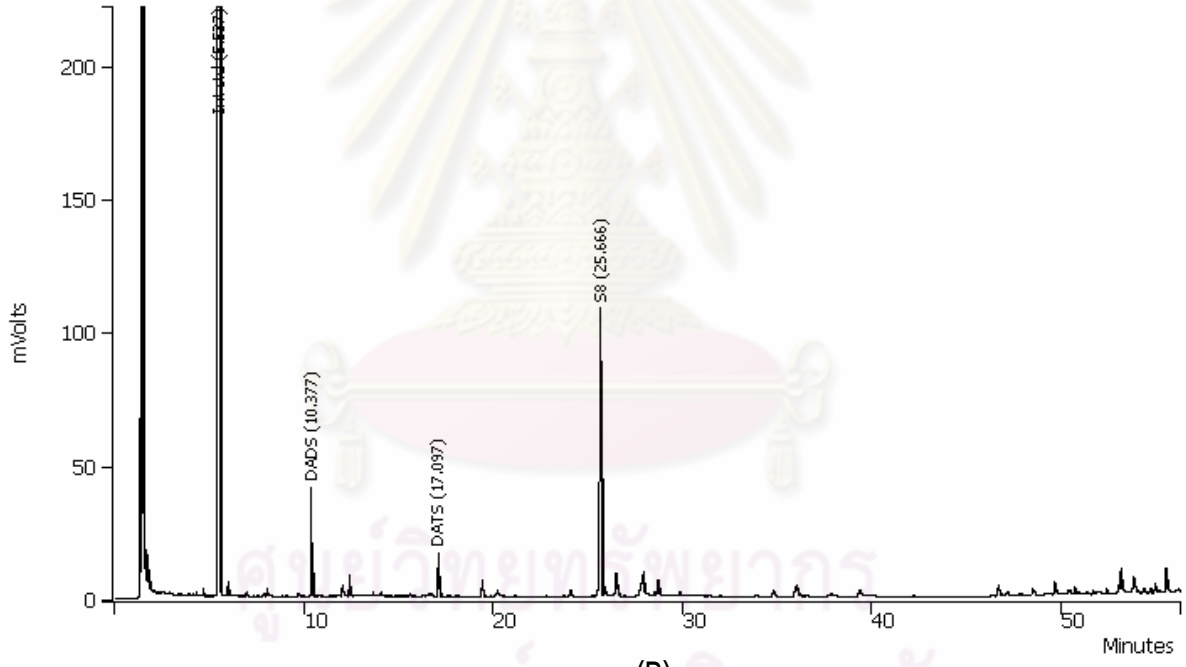


(B)

Figure D5 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 0.8% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 160±5°C for the 1st (A) and 2nd (B) experiments are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)

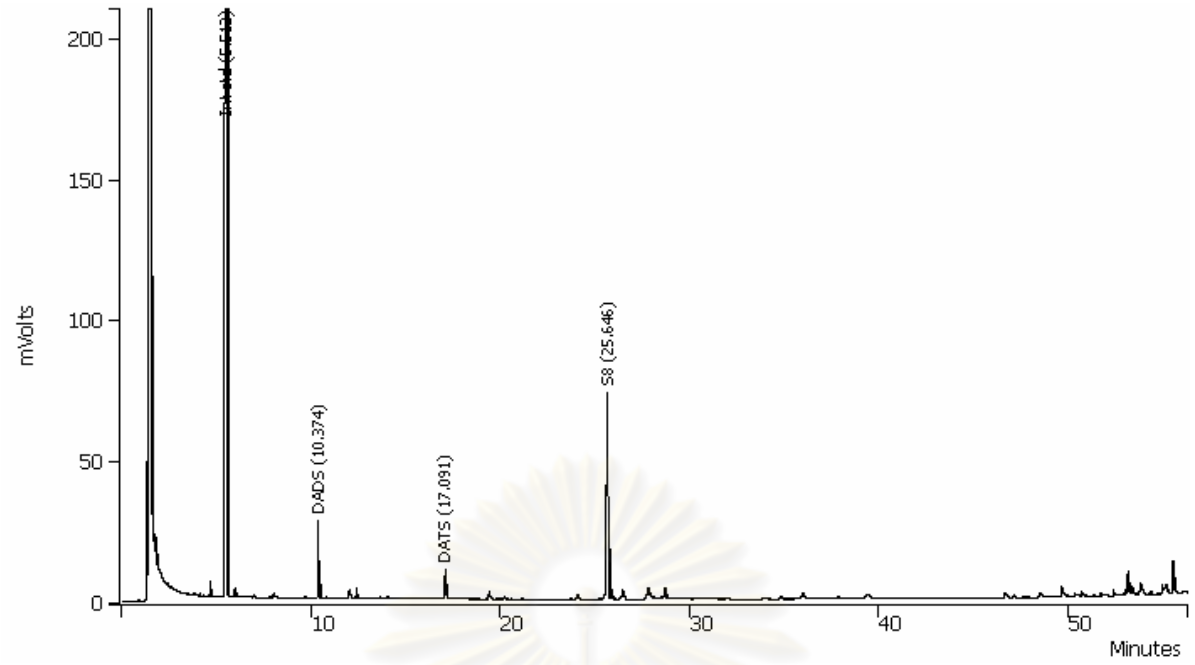


(A)

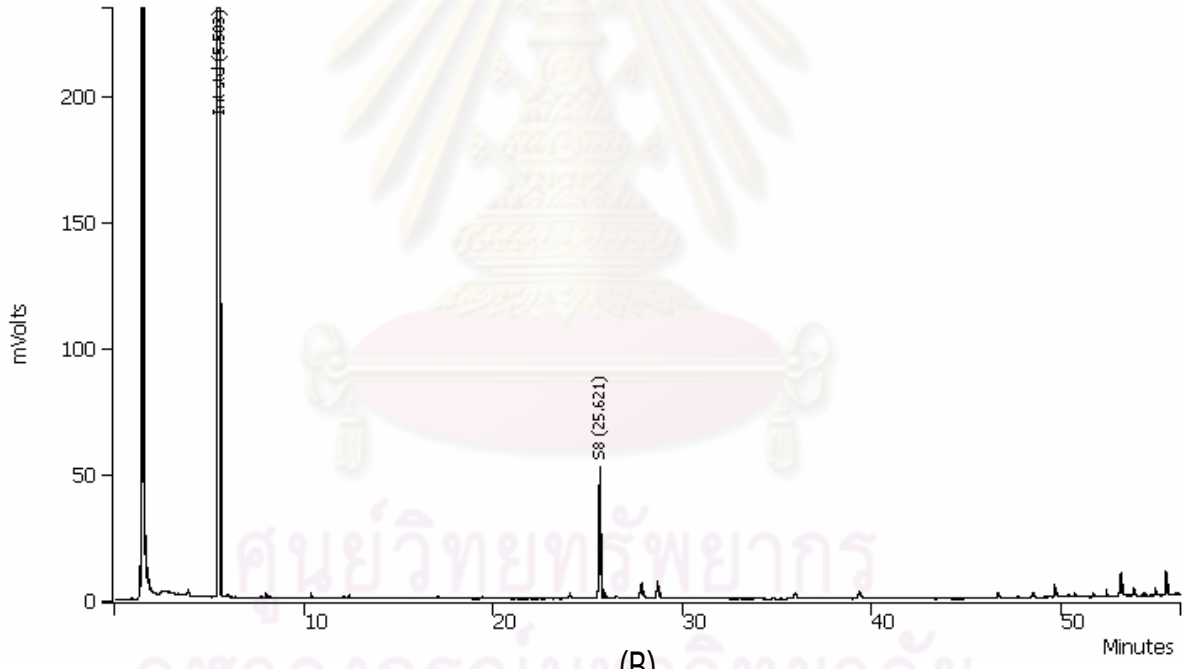


(B)

Figure D6 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 0.8% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 180±5°C for the 1st (A) and 2nd (B) experiments are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)

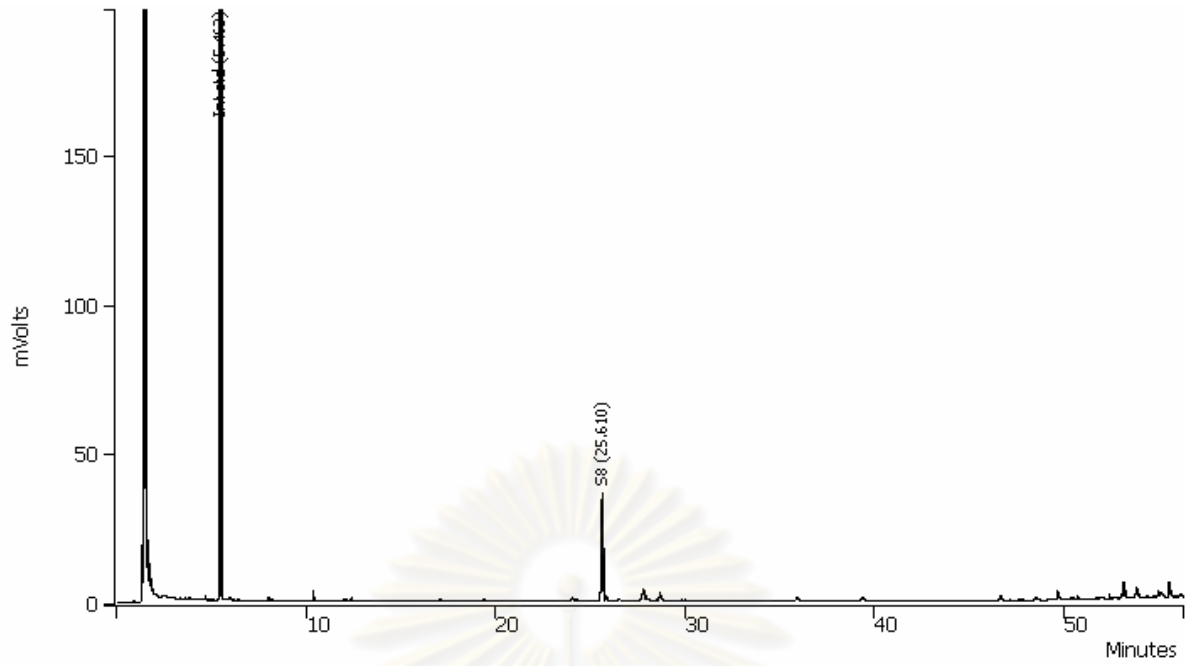


(A)

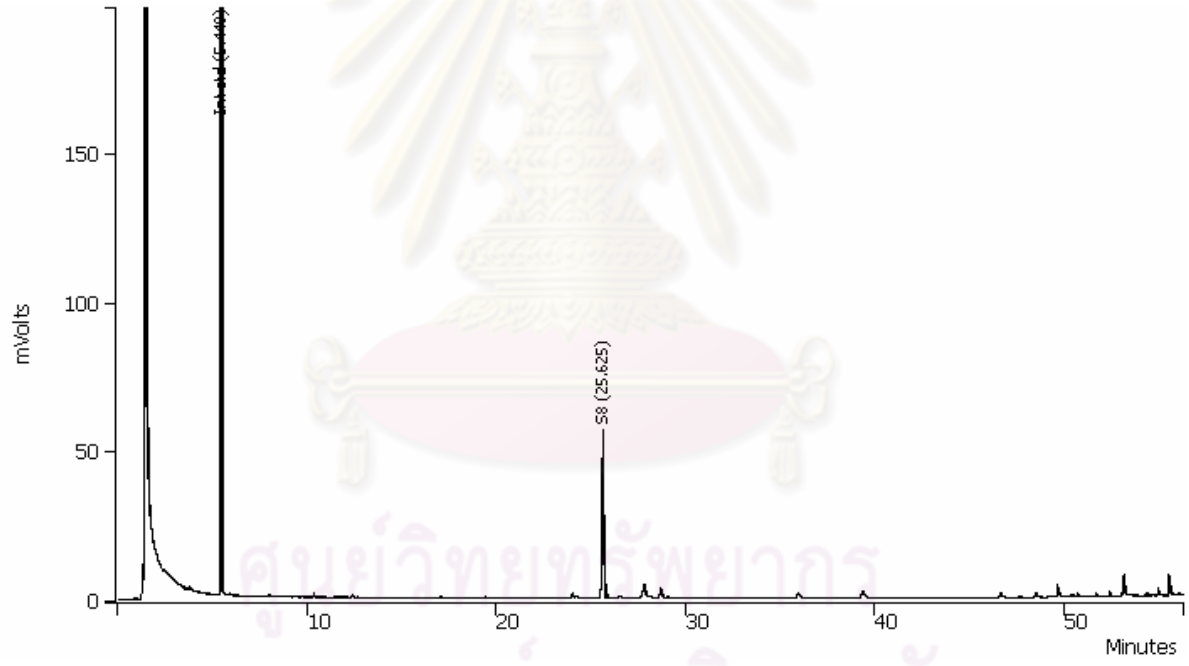


(B)

Figure D7 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 0.8% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 200±5°C for the 1st (A) and 2nd (B) experiment are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)

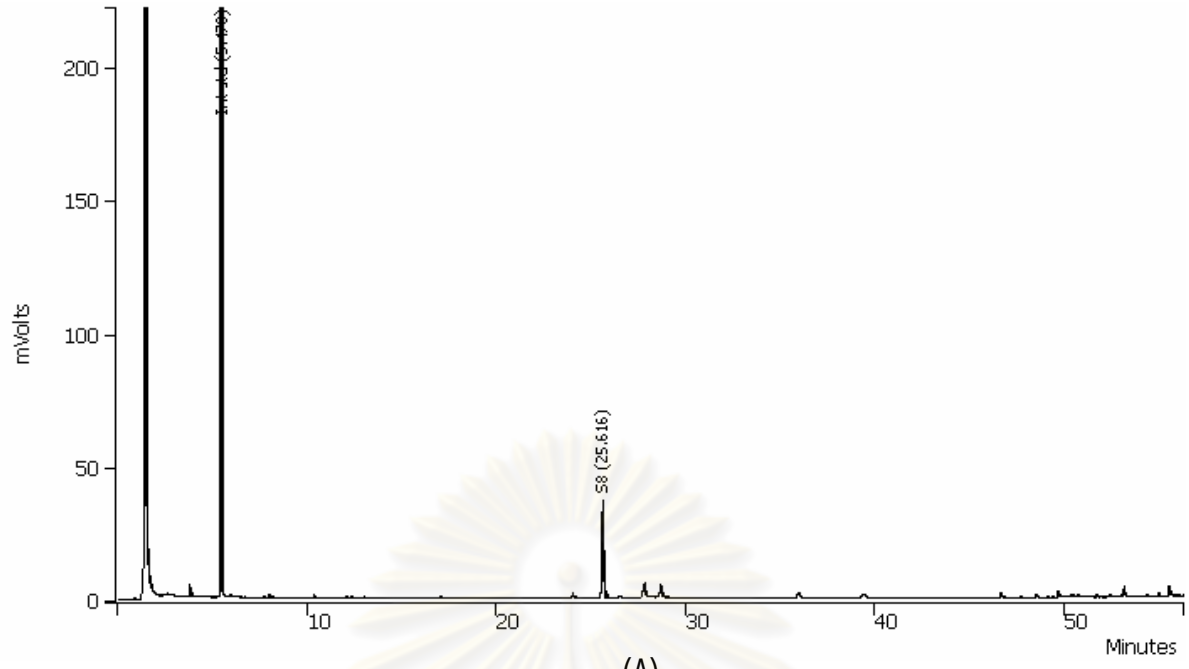


(A)

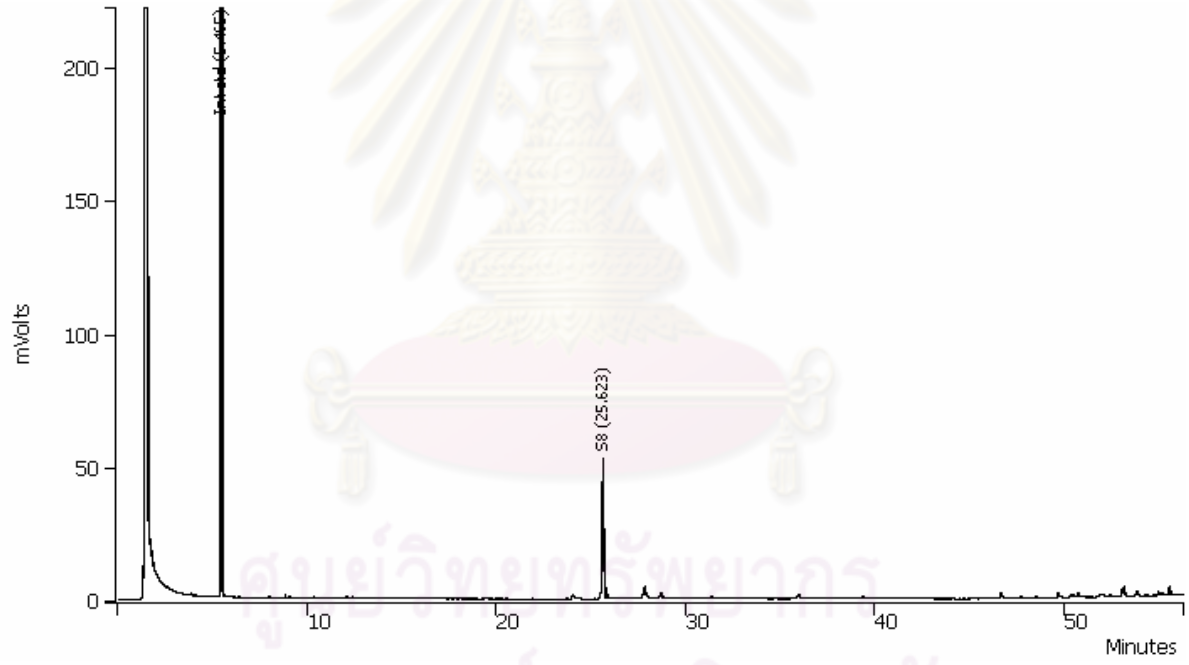


(B)

Figure D8 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 1% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 160±5°C for the 1st (A) and 2nd (B) experiment are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)

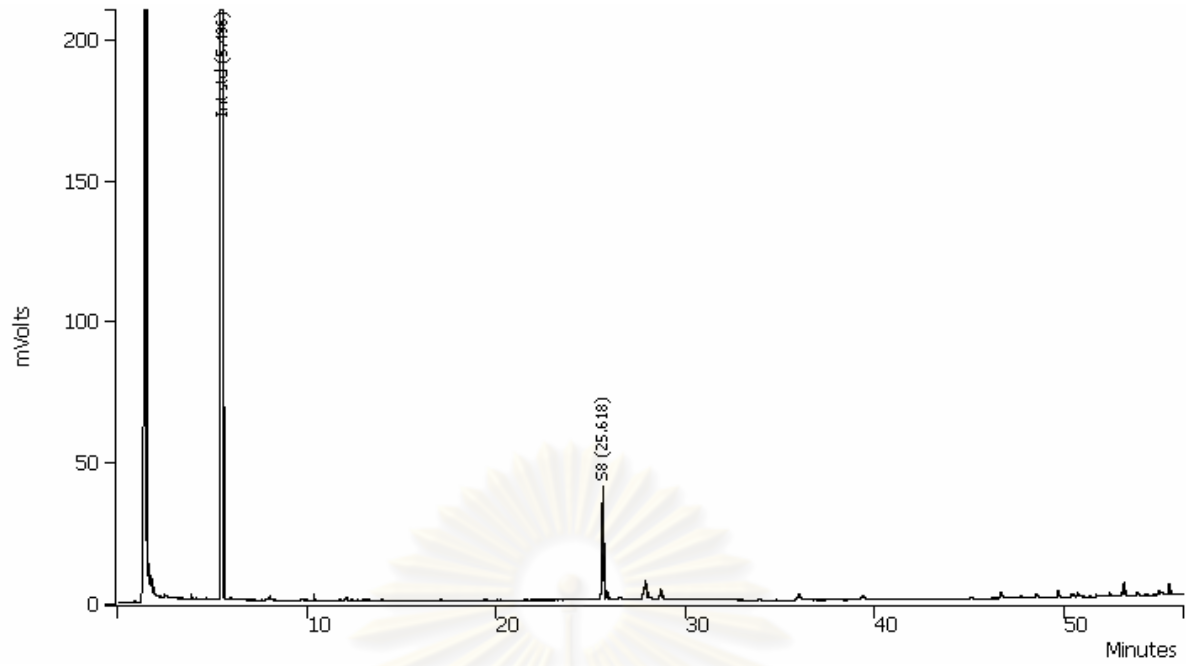


(A)

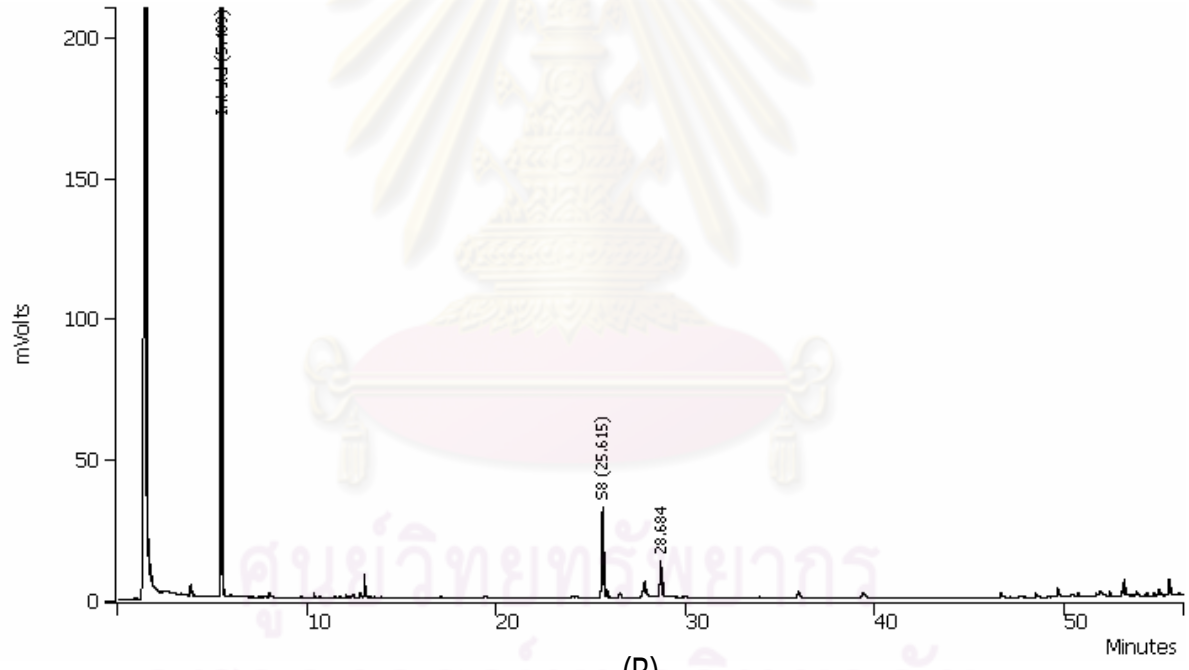


(B)

Figure D9 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 1% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 180±5°C for the 1st (A) and 2nd (B) experiment are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)



(A)



(B)

Figure D10 Typical chromatograms of garlic oil microcapsules prepared from the feed emulsion that contains 0.2:1 oil-to-maltodextrin ratio and 1% w/w Tween®20 in 20 g/dL maltodextrin. The spray drying was carried out at an inlet temperature of 200±5°C for the 1st (A) and 2nd (B) experiment are shown in this figure. The initial on the chromatograms indicates the compounds as below: Dialyll disulfides (DADS) and cyclic sulfurs (S_8)

Table D15 Inhibition ability of garlic oil emulsion heated at various temperatures (70°C, 80°C and 90°C) for 0-25 minutes against *Staphylococcus aureus* ATCC 25923

Heating time (min)	Inhibition zone diameter (cm)					
	70°C		80°C		90°C	
	First	Second	First	Second	First	Second
0.5	0.800	0.700	0.900	1.000	-	-
1.0	0.550	0.650	0.800	0.800	0.420	0.410
1.5	0.500	0.500	0.800	0.700	0.400	0.400
2.0	0.480	0.540	0.800	0.700	0.350	0.400
2.5	0.450	0.520	0.800	0.700	0.350	0.350
3.0	0.400	0.510	0.800	0.700	0.300	0.320
3.5	0.500	0.400	0.700	0.700	0.200	0.300
4.0	0.500	0.350	0.700	0.600	0.200	0.300
4.5	0.400	0.300	0.700	0.700	0.300	0.200
5.0	0.300	0.450	0.700	0.600	0.250	0.100
5.5	0.300	0.450	0.600	0.600	0.300	0.200
6.0	0.300	0.450	0.600	0.500	0.300	0.200
6.5	0.500	0.300	0.500	0.500	0.200	0.200
8.5	0.500	0.400	0.500	0.500	0.250	0.300
10.0	0.450	0.300	0.500	0.500	0.250	0.250
13.0	0.400	0.200	0.400	0.400	0.240	0.220
15.0	0.400	0.210	0.400	0.400	0.250	0.210
20.0	0.300	0.100	0.400	0.400	0.220	0.220
25.0	-	-	0.300	0.300	0.200	0.200

Table D16 Inhibition ability of garlic oil emulsion heated at various temperatures (70°C, 80°C and 90°C) for 0-25 minutes against *Salmonella* Typhimurium ATCC 13311.

Heating time (min)	Inhibition zone diameter (cm)					
	70°C		80°C		90°C	
	First	Second	First	Second	First	Second
0.5	0.450	0.400	0.500	0.500	0.400	0.450
1.0	0.400	0.350	0.400	0.500	0.400	0.400
1.5	0.350	0.300	0.400	0.400	0.300	0.350
2.0	0.350	0.350	0.400	0.400	0.350	0.300
2.5	0.320	0.300	0.400	0.500	0.300	0.320
3.0	0.300	0.400	0.400	0.400	0.250	0.300
3.5	0.250	0.200	0.300	0.400	0.200	0.220
4.0	0.200	0.200	0.300	0.300	0.100	0.100
4.5	0.100	0.100	0.400	0.300	0.100	0.100
5.0	0.100	0.100	0.400	0.400	0.100	0.100
5.5	0.100	0.100	0.500	0.400	0.100	0.100
6.0	0.100	0.100	0.300	0.300	0.100	0.100
6.5	0.000	0.000	0.300	0.300	0.000	0.000
8.5	0.000	0.000	0.300	0.300	0.000	0.000
10.0	0.000	0.000	0.400	0.300	0.000	0.000
13.0	0.000	0.000	0.500	0.400	0.000	0.000
15.0	0.000	0.000	0.300	0.300	0.000	0.000
20.0	0.000	0.000	0.200	0.200	0.000	0.000
25.0	-	-	0.200	0.100	0.400	0.450

Table D17 Inhibition ability of garlic oil emulsion heated at various temperatures (70°C, 80°C and 90°C) for 0-25 minutes against *Escherichia coli* ATCC 25922.

Heating time (min)	Inhibition zone diameter (cm)					
	70°C		80°C		90°C	
	First	Second	First	Second	First	Second
0.5	0.400	0.400	0.500	0.600	-	-
1.0	0.400	0.400	0.500	0.500	0.600	0.500
1.5	0.400	0.400	0.300	0.500	0.500	0.500
2.0	0.400	0.400	0.400	0.500	0.500	0.500
2.5	0.400	0.400	0.400	0.500	0.500	0.500
3.0	0.300	0.400	0.400	0.300	0.500	0.500
3.5	0.300	0.300	0.400	0.300	0.400	0.500
4.0	0.300	0.300	0.400	0.300	0.400	0.500
4.5	0.400	0.300	0.300	0.400	0.400	0.400
5.0	0.400	0.300	0.100	0.400	0.400	0.400
5.5	0.300	0.300	0.100	0.400	0.300	0.300
6.0	0.300	0.200	0.100	0.400	0.300	0.300
6.5	0.300	0.300	0.000	0.300	0.300	0.300
8.5	0.400	0.200	0.200	0.200	0.300	0.200
10.0	0.300	0.200	0.200	0.100	0.300	0.300
13.0	0.300	0.200	0.200	0.200	0.300	0.200
15.0	0.300	0.100	0.000	0.100	0.200	0.100
20.0	0.100	0.100	0.000	0.000	0.000	0.000
25.0	-	-	0.000	0.000	0.000	0.000

Table D18 The average width of inhibition zone of garlic oil microcapsules in water against *Staphylococcus aureus* ATCC 25923.

Time	Width of inhibition zone (cm)
5 min	0.433±0.11
10 min	0.333±0.06
15 min	0.367±0.06
20 min	0.333±0.06
25 min	0.333±0.06
50 min	0.333±0.06
1 hour	0.467±0.06
2 hours	0.433±0.06
3 hours	0.433±0.05
4 hours	0.467±0.06
5 hours	0.467±0.06
6 hours	0.500±0.00
8 hours	0.433±0.06
10 hours	0.433±0.06
12 hours	0.433±0.06
24 hours	0.467±0.06
48 hours	0.433±0.06

* The measurement was done in triplicate.

Table D19 The average sensory scores for salad dressing containing garlic oil microcapsules and control salad dressing stored at 25^{0}C for 1 to 5 days

Day	Sample	Color*	Odor*	Smoothness*	Viscosity*	Acceptability*
1	R	1.33±0.55	1.57±0.73	4.63±1.10	4.53±0.86	5.17±0.87
	S	2.58±0.12	6.37±0.19	3.38±0.12	5.75±0.02	2.50±0.05
3	R	1.33±0.55	1.57±0.73	4.63±1.10	4.53±0.86	5.17±0.87
	S	3.28±0.02	6.65±0.07	3.28±0.07	5.52±0.07	2.32±0.12
5	R	1.33±0.55	1.57±0.73	4.63±1.10	4.53±0.86	5.17±0.87
	S	4.40±0.33	6.53±0.05	4.40±0.19	5.38±0.21	2.02±0.02

R was control dressing and S was Salad dressing containing garlic oil microcapsules.
* Average score from 50 assessors.

Appendix E

Statistics

Table E1 Analysis of variance of oil droplet size D[3,2] of garlic containing emulsion of 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin ratio in 0.6%w/wTween®20 in 20 g/dL maltodextrin solution stored at 4°C

Tests of Between-Subjects Effects					
Dependent Variable:size					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.031[a]	5	.006	12.540	.000
Intercept	1.277	1	1.277	2564.784	.000
ratio	.007	2	.004	7.431	.002
rpm	.023	2	.011	23.085	.000
time	.001	1	.001	1.669	.206
Error	.015	30	.000		
Total	1.323	36			
Corrected Total	.046	35			

a. R Squared = .676 (Adjusted R Squared = .622)

Table E2 Analysis of variance of oil droplet size D[3,2] of garlic oil emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin ratio in 0.8%w/w Tween®20 in 20 g/dL maltodextrin solution stored at 4°C

Tests of Between-Subjects Effects

Dependent Variable:size

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.045[a]	5	.009	17.607	.000
Intercept	1.238	1	1.238	2439.882	.000
ratio	.007	2	.004	7.174	.003
rpm	.037	2	.018	36.044	.000
time	.001	1	.001	1.600	.216
Error	.015	30	.001		
Total	1.298	36			
Corrected Total	.060	35			

a. R Squared = .746 (Adjusted R Squared = .703)

Table E3 Analysis of variance of oil droplet size D[3,2] of garlic oil emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin ratio in 1%w/w Tween®20 in 20 g/dL maltodextrin solution stored at 4°C

Tests of Between-Subjects Effects

Dependent Variable:size

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.078[a]	5	.016	12.753	.000
Intercept	1.338	1	1.338	1097.582	.000
Ratio	.019	2	.010	7.850	.002
Rpm	.052	2	.026	21.149	.000
Time	.007	1	.007	5.764	.023
Error	.037	30	.001		
Total	1.453	36			
Corrected Total	.114	35			

a. R Squared = .680 (Adjusted R Squared = .627)

Table E4 Analysis of variance of oil droplet size D[3,2] of garlic oil emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 0.6%w/w Tween®20 in 20 g/dL maltodextrin compared with that in distilled water stored at 4°C

Ratio of 0.1:1;

ANOVA

Droplet tsize

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	1	.000	.061	.810
Within Groups	.014	10	.001		
Total	.014	11			

Ratio of 0.15:1;

ANOVA

Droplet size

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	1	.001	1.993	.188
Within Groups	.005	10	.001		
Total	.006	11			

Ratio of 0.2:1;

ANOVA

Droplet size

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	1	.001	.770	.401
Within Groups	.009	10	.001		
Total	.010	11			

Table E5 Analysis of variance of oil droplet size D[3,2] of garlic oil emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 0.8%w/w Tween®20 in 20 g/dL maltodextrin compared with that in distilled water stored at 4°C

Ratio of 0.1:1;

ANOVA

Droplet size

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.006	1	.006	.183	.678
Within Groups	.336	10	.034		
Total	.342	11			

Ratio of 0.15:1;

ANOVA

Droplet size

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	1	.000	.194	.669
Within Groups	.009	10	.001		
Total	.009	11			

Ratio of 0.2:1;

ANOVA

Droplet size

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	1	.000	.445	.520
Within Groups	.009	10	.001		
Total	.010	11			

Table E6 Analysis of variance of oil droplet size D[3,2] of garlic oil emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 1%w/w Tween®20 in 20 g/dL maltodextrin compared with that in distilled water stored at 4°C

Ratio of 0.1:1;

ANOVA

Droplet tsize

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.002	1	.002	1.547	.242
Within Groups	.010	10	.001		
Total	.012	11			

Ratio of 0.15:1;

ANOVA

Droplet size

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.003	1	.003	.382	.550
Within Groups	.082	10	.008		
Total	.085	11			

Ratio of 0.2:1;

ANOVA

Droplet size

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	1	.000	.022	.885
Within Groups	.118	10	.012		
Total	.118	11			

Table E7 Analysis of variance of garlic oil microcapsule size D[3,2]. The microcapsules were prepared from an emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 0.6%, 0.8% and 1%w/w Tween®20 in 20 g/dL maltodextrin and spray-dried at various inlet air temperatures (120°C, 160°C and 180°C).

Tests of Between-Subjects Effects

Dependent Variable: Powder size D[3,2]

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	21.773[a]	5	4.355	6.881	.001
Intercept	2834.206	1	2834.206	4478.694	.000
tweenconc	2.777	2	1.389	2.194	.140
inlettemp	18.996	3	6.332	10.006	.000
Error	11.391	18	.633		
Total	2867.370	24			
Corrected Total	33.164	23			

a. R Squared = .657 (Adjusted R Squared = .561)

Table E8 Analysis of variance of moisture (%w/w) of garlic oil microcapsule of 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 0.6%, 0.8% and 1%w/w Tween®20 in 20 g/dL maltodextrin at various inlet air temperature including 120°C, 160°C and 180°C

Tests of Between-Subjects Effects

Dependent Variable: moisture

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.160a	5	2.232	20.105	.000
Intercept	16.157	1	16.157	145.542	.000
tweenconc	.090	2	.045	.405	.673
inlettemp	11.070	3	3.690	33.239	.000
Error	1.998	18	.111		
Total	29.315	24			
Corrected Total	13.158	23			

a. R Squared = .848 (Adjusted R Squared = .806)

Table E9 Analysis of variance of water activity (A_w). The microcapsules were prepared from an emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 0.6%, 0.8% and 1%w/w Tween®20 in 20 g/dL maltodextrin and spray-dried at various inlet air temperatures (120°C, 160°C and 180°C)

Tests of Between-Subjects Effects

Dependent Variable: A_w

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.050[a]	5	.010	12.799	.000
Intercept	.416	1	.416	531.035	.000
tweenconc	.007	2	.003	4.173	.032
inlettemp	.044	3	.015	18.549	.000
Error	.014	18	.001		
Total	.480	24			
Corrected Total	.064	23			

a. R Squared = .780 (Adjusted R Squared = .719)

Table E10 Analysis of variance of total oil (%w/w). The microcapsules were prepared from an emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 0.6%, 0.8% and 1%w/w Tween®20 in 20 g/dL maltodextrin and spray-dried at various inlet air temperatures (120°C, 160°C and 180°C)

Tests of Between-Subjects Effects

Dependent Variable: Total oil

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.441[a]	5	1.688	7.178	.001
Intercept	130.419	1	130.419	554.528	.000
tweenconc	2.861	2	1.430	6.082	.010
inlettemp	5.580	3	1.860	7.909	.001
Error	4.233	18	.235		
Total	143.093	24			
Corrected Total	12.674	23			

a. R Squared = .666 (Adjusted R Squared = .573)

Table E11 Analysis of variance of bulk density ($g \cdot cm^{-1}$). The microcapsules were prepared from an emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 0.6%, 0.8% and 1%w/w Tween®20 in 20 g/dL maltodextrin and spray-dried at various inlet air temperatures (120°C, 160°C and 180°C)

Tests of Between-Subjects Effects

Dependent Variable: Bulk density

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.018[a]	5	.004	.800	.564
Intercept	5.783	1	5.783	1271.379	.000
tweenconc	.001	2	.001	.110	.896
inlettemp	.017	3	.006	1.259	.318
Error	.082	18	.005		
Total	5.883	24			
Corrected Total	.100	23			

a. R Squared = .182 (Adjusted R Squared = -.046)

Table E12 Analysis of variance of solubility (%w/w). The microcapsules were prepared from an emulsion containing 0.1:1, 0.15:1 and 0.2:1 of oil to maltodextrin in 0.6%, 0.8% and 1%w/w Tween®20 in 20 g/dL maltodextrin and spray-dried at various inlet air temperatures (120°C, 160°C and 180°C)

Tests of Between-Subjects Effects

Dependent Variable: Solubility

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.468a	5	.694	1.252	.390
Intercept	111813.440	1	111813.440	201880.930	.000
tweenconc	2.628	2	1.314	2.373	.174
inlettemp	.840	3	.280	.505	.693
Error	3.323	6	.554		
Total	111820.232	12			
Corrected Total	6.791	11			

a. R Squared = .511 (Adjusted R Squared = .103)

VITA

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