EXTRACTION AND RECOVERY OF PHORBOL ESTERS FROM JATROPHA PRESSED SEEDS BY SURFACTANT SOLUTION

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การสกัดและการนำกลับคืนสารฟอร์บอลเอสเทอร์จากกากเมล็ดสบู่ดำที่ผ่านกระบวนการหีบน้ำมันโดยสารละลายลดแรงตึงผิว

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ณภัสนันท์ พสุการัชต์ชัย : การสกัดและการนำกลับคืนสารฟอร์บอลเอสเทอร์จากกากเมล็ดสบู่ดำที่ผ่านกระบวนการหีบน้ำมันโดยสารละลายลดแรงตึงผิว (EXTRACTION AND RECOVERY OF PHORBOL ESTERS FROM JATROPHA PRESSED SEEDS BY SURFACTANT SOLUTION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. จันทรา ทองคำเภา, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. ศีลาวุธ ดำรงศิริ, 147 หน้า.

สารฟอร์บอลเอสเทอร์ (PEs) พบได้ทั้งในน้ำมันสบู่ดำและกากเมล็ดสบู่ดำที่ผ่านการกระบวนหีบน้ำมัน ซึ่ง PEs มีคุณสมบัติในการกำจัดศัตรูพืช และมีความเป็นไปได้ในการออกฤทธิ์ทางยา การสกัด PEs โดยทั่วไปใช้เมทานอล ทั้งนี้การใช้เมทานอลต้องคำนึงถึงความปลอดภัยต่อสุขภาพและความเสี่ยงอื่น ๆ ดังนั้น สารละลายลดแรงตึงผิวที่มีความเป็นพิษต่ำปลอดภัยต่อสิ่งแวดล้อม และราคาถูก จึงเป็นวิธีที่น่าสนใจในการใช้สกัดแทนตัวทำละลายระเหยได้ จากผลการศึกษาพบว่า ในระบบสารละลายลดแรงตึงผิวแบบปกติ PEs มีพฤติกรรมคล้ายคลึงสารอินทรีย์โมเลกุลใหญ่ที่ค่อนข้างมีขั้ว ซึ่งละลายอยู่ในชั้นพาไลเซดด้านนอกของไมเซลล์ สารลดแรงตึงผิวชนิดไม่มีประจุที่มีโครงสร้างของเอทิลีนออกไซด์สูง มีความสามารถในการละลาย PEs ได้ดีกว่าสารลดแรงตึงผิวชนิดประจุลบ ประสิทธิภาพการสกัด PEs จากกากเมล็ดสบู่ดำสูงที่สุดถึง 85% ได้จากการใช้สารลดแรงตึงผิวลอเรท-12 ความเข้มข้น 9.4% โดยมวลต่อปริมาตร อัตราส่วนระหว่างกากสบู่ดำและสารละลายที่ 100 กรัมต่อลิตร ที่ความเร็วรอบ 1000 รอบต่อนาที ระยะเวลา 40 นาที ซึ่งมีประสิทธิภาพไม่แตกต่างจากการสกัดด้วยเมทานอลภายใต้สภาวะเดียวกัน สารละลายหลังจากแยกกากสบู่ดำออกจะนำมาผ่านการแยกชั้นและทำให้เข้มข้นขึ้นด้วยเทคนิคการเพิ่มอุณหภูมิตามจุดขุ่น (cloud point) โดยการเติมเกลือช่วยลดอุณหภูมิในการแยกสาร พบว่าประจุลบมีผลต่อการลดอุณหภูมิมากกว่าประจุบวก โดยประจุฟอสเฟตไตรวาเลนท์มีประสิทธิภาพดีที่สุดในกลุ่มประจุลบ เมื่อทำการทดลองจริงกับสารสกัด PEs พบว่า เกลือโซเดียมซัลเฟตให้ประสิทธิภาพการนำกลับคืน PEs สูงที่สุดถึง 91% ที่อุณหภูมิ 40 องศาเซลเซียส แต่ข้อจำกัดของการแยกสารด้วยวิธีนี้คือ PEs จะอยู่ในสารละลายลอเรท-12 ดังนั้นการนำ PEs ไปใช้ประโยชน์ต้องคำนึงถึงผลกระทบที่เกิดจากลอเรท-12 ด้วยการทดลองเบื้องต้นพบว่าตัวทำละลายมีขั้ว เช่น บิวทานอล และไดคลอโรมีเทน สามารถสกัด PEs ได้ดีกว่าตัวทำละลายไม่มีขั้วเฮกเซน ด้านการเก็บรักษาวัตถุดิบก่อนการสกัดนั้น ทั้งน้ำมันสบู่ดำและกากเมล็ดสบู่ดำควรเก็บภายใต้สภาวะที่เหมาะสม โดยหลีกเลี่ยงอุณหภูมิสูง และการสัมผัสแสง เนื่องจาก PEs จะสลายตัวได้เร็วขึ้นและควรรีบทำการสกัดสารทันทีหลังจากเมล็ดสบู่ดำผ่านการหีบน้ำมันแล้ว

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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NAPHATSARNAN PHASUKARRATCHAI: EXTRACTION AND RECOVERY OF PHORBOL ESTERS FROM JATROPHA PRESSED SEEDS BY SURFACTANT SOLUTION. ADVISOR: ASST. PROF. CHANTRA TONGCUMPOU, Ph.D., CO-ADVISOR: SEELAWUT DAMRONGSIRI, Ph.D., 147 pp.

Phorbol esters (PEs) are bio-pesticide and potentially applied for health treatment, found in jatropha oil and pressed seeds. PEs is generally extracted by methanol; safety and health risk becomes concerns. Thus, surfactant solutions are introduced as alternative solvent. The results indicate that PEs act as a large polar organic molecule and locate in outer palisade of a surfactant micelle. Nonionic surfactants with high EON have found higher extraction efficiency than those of anionic surfactants. Laureth-12 yields the highest efficiency 85%, which is comparable with methanol, under the optimal condition at 9.4% of laureth-12, 100 g/L of solid to liquid ratio, 1000 rpm of agitation speed, and 40 min of extraction time. In recovery PEs, cloud point (CP) phase separation technique has been conducted; the extract PEs-in the laureth-12 solution has CP >100°C. Anion of electrolytes showed higher efficiency than cation, and trivalent anionic was the best for lowering CP. However, 5.9% of Na_2SO_4 show the best normalized score of factors with, 91% of PEs recovery at 40°C. The limitation of CP extraction is that PEs and laureth-12 were extracted together, thus further extraction to separate PEs from surfactant is needed. The preliminary result for this extraction shows that polar solvent i.e. butanol and dichloromethane perform better than nonpolar solvent (hexane). The jatropha pressed seeds and crude oil, PEs raw material, should keep under suitable condition, to avoid high temperature and light exposure, which cause highly PEs degraded. PEs should be extracted soon after harvest and pressed the seeds for oil production.

Field of Study:	Environmental Management	Student's Signature	.....
		Advisor's Signature	.....
Academic Year:	2015	Co-Advisor's Signature	.....

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ABBREVIATIONS

Abbreviations	*Full name*
C-chain	Carbon chain
CMC	Critical micellar concentration
CP	Cloud point
d	Day
EO	Ethylene oxide
EON	Ethylene oxide number
g	Gram
HLB	Hydrophile-lipophlie balance
IFT	Interfacial tension
JCO	Jatropha crude oil
JPS	Jatropha pressed seeds
kg	Kilogram
L	Liter
LAE	Linear alcohol ethoxylate
mg	Milligram
min	Minute
mL	milliliter
mmol	Millimole
mol	Mole
MSR	Molar solubilization ratio
PEs	Phorbol esters
S:L	Solid to liquid ratio
SDS	Sodium dodecyl sulfate
SLES	Sodium lauryl ether sulfate

CHAPTER I
INTRODUCTION

1.1. Motivations

Phorbol esters (PEs), plant-derive organic compound, are found in Euphobiaceae family such as *Croton* sp. and *Jatropha* sp. [1, 2]. These compounds have been considered as the suspected carcinogens and severe irritants when absorbed into the body [2]. However, some positive applications of PEs were discovered for agricultural and pharmaceutical aspect. The methanol enriched with PEs extracted from *J. curcas* oil was observed to efficiently control the intermediate snail hosts and larvae of schistosomes [3], the mite [4], and the third instar larvae of *Spodoptera frugiperda* [5]. Moreover, PEs were found potential to be used for leukemia remedy [6] and used as the initial substance to synthesis prostratin for HIV treatment [7].

Jatropha curcas L. plant is interesting as a source of alternative fuel because its seeds contain high amount of non-edible oil. The popular process for extracting the oil from seeds is a screw press, approximately one liter of pressed oil is derived from four kilograms of seeds [2]. The pressed seeds, a by-product of oil recovery process about three kilograms per one liter of oil yield, contain high amount of protein and are expected to be a raw material for animal feed production as if it pass the detoxification process. PEs contain both in the oil (1.11 mg as TPA g^{-1}) and the pressed seeds (1.45 mg as TPA g^{-1}) [8]. For the oil, PEs is disappeared during the tranesterification process to produce biodiesel [9]. For the pressed seeds, the main utilization is used as fertilizer in agriculture area. In order to apply the pressed seeds for animal feeding, the PEs removal by a base reaction with alcohol washing yields the high efficiency [10, 11]. The heat treatment only was observed as low competence [12].

The separation of PEs from *J. curcas* oil and pressed seeds would make all *J. curcas*'s composition (oil, meal, and PEs) to be fully utilized which considered high efficiency on resources utilization. Accordingly, several techniques have been

developed to remove PEs from the Jatropha oil and pressed seed. Mostly, PEs removal techniques are studied on volatile organic solvent [13, 14] and adsorbent [11]. Previous studies have revealed that methanol yields the highest PEs extraction efficiency [12, 14, 15]. However, most volatile organic compounds cause high risk in explosive and toxic to health; moreover, the operation has to be in a close system. In addition, water alone cannot extract PEs from the oil or from the pressed seeds because of the hydrophobicity of PEs [16]. Thus, an aqueous-based surfactant solution, which non-volatile and environmental safe, was introduced as an alternative solvent to extract biochemical from plants [17-19].

Generally, surfactant molecules have hydrophilic and lipophilic components in their structure, which enable them to reduce the interfacial tension between two immiscible phases [20]. At concentrations above the critical micelle concentration (CMC), surfactants can form micelles and cause less polar or nonpolar compounds to solubilize within the micelle [20]. Many researchers have observed that the solubilization of low-water-soluble organic compounds can be enhanced in water in the presence of a nonionic surfactant [21-24]. An increase of the hydrophilic part in surfactant molecules with the same hydrophobic part can increase the solubilization of polar compounds; in contrast, nonpolar compound solubilization is dependent on the hydrophobic component of the surfactant [20, 21]. Dehydration of a nonionic surfactant system during a temperature increase and electrolyte addition to an anionic surfactant system can enhance nonpolar compound solubilization [20, 21]. Moreover, mixing anionic and nonionic surfactants can prevent nonionic adsorption and partitioning into an organic oil [25], and the resulting mixture has a synergistic effect on solubilization enhancement [26-28]. Water-based surfactant solutions can potentially extract PEs from jatropha pressed seed [19]. However, the solubilization of PEs is limited.

The extraction factors should be considered in order to yield high efficiency both in the solvent selection and extraction condition. For surfactant selection, the properties of surfactant and the structure of target compounds should be considered [20, 29, 30]. The physical condition plays important role in the extraction process

related to mass transfer such as solid to liquid ratio, temperature, mixing speed, and contact time [31, 32].

In order to recovery target compounds from solvent, volatile solvents are generally evaporated out. In contrast, surfactant cannot be separated from target compound easily. However, the concentration and recovery of target compound can possibly be done by cloud point technique [17, 18]. Theoretically, solution of polyethylene oxide nonionic surfactant will be separated in two phases known as surfactant-rich phase and dilution phase at above a certain temperature. This phenomenon is called “Cloud point”. As a result of phase separation, solubilizates or extracted target will also be concentrated in the rich phase [20, 33]. In addition, the low hydrophile-lipophile balance (HLB) surfactant and electrolyte additive can adjusted the cloud point temperate [34-36] for PEs concentrating and ready to use for further purification.

Regarding to potential benefits of PEs, the degradation of compounds should be better understood. Autoxidation was believed to be a major mechanism for PEs degradation in room temperature [37]. Moreover, these compounds were found to be degraded under sunlight exposure [38]. They were also found to biologically degraded by bacterial activity under facultative condition (fermentation) [39-41] and white-rot-fungi under aerobic condition [42]. However, the natural degradation of PEs that is the overall degradation in raw materials (Jatropha crude oil and pressed seeds) is not yet clarified.

Thus, the aim of this research is to extract and recovery PEs from the Jatropha pressed seeds. The PEs solubilization was investigated with *J. curcas* oil to select some suitable surfactants. In addition, the effect of surfactant structures properties on PEs solubilization in aqueous phase was evaluated. In the PEs extraction part, the chemical factors of surfactants and the physical factors were evaluated for the significant effect to determine the optimal condition. Then, the PEs extracted solution will be concentrated for the further application. To fulfill Jatropha oil recovery process management, the natural degradation of PEs in the oil as well as in pressed seeds under the different storage conditions and storage time was studied.

1.2. Objectives

To extract and recovery phorbol esters (PEs) from Jatropha pressed seeds by using surfactant solution.

Sub-objectives:

1.2.1. To investigate PEs solubilization in Jatropha crude oil for screening suitable surfactants aqueous-based solutions.

1) To study the effect of properties and structures of surfactant including of nonionic and anionic type, ethylene oxide number, carbon chain length, and hydrophile-lipophile-balance (HLB) value.
2) To study the effect of surrounding system change in the surfactant solution including of electrolyte adding for anionic surfactants and temperature change for nonionic surfactant.

1.2.2. To investigate the optimal factors for PEs extraction from Jatropha pressed seeds.

1) To study the effect of chemical factors including of surfactants properties and concentration.
2) To study the effect of physical factors including of grain size, solid-liquid ratio, speed of mixing, and contact time.

1.2.3. To investigate the PEs recovery from the selected surfactant solution by liquid-liquid extraction, temperature changing, and electrolyte addition.

1.2.4. To study the natural degradation of PEs in Jatropha crude oil and pressed seeds during storage under different condition (light exposure, light source, temperature and sample pretreatment).

1.3. Hypotheses

1) Solubilization of PEs increases with an increase of ethylene oxide number in surfactant molecule.
2) Solubilization of PEs from Jatropha oil to surfactant solutions increase with an increase of carbon-chain length in surfactant molecule.
3) Solubilization of PEs increases with an increase of HLB of surfactants.

4) Physical properties of extraction such as a solid to liquid ration, a speed of mixing and the extraction time affect the efficiency of PEs extraction.
5) The temperature requirement for separating PEs from the surfactant solution can be manipulated by electrolyte addition.
6) PEs (in Jatropha oil and pressed seeds) are degraded faster under the higher light intensity.

1.4. Scope

There are four parts in this study. The first part is to investigate the effect of surfactant structure on PEs solubilization in order to screen for surfactant for the next part. Jatropha oil is selected as PEs source for solubilization part. The second part aims to investigate the proper systems and the factors affected on PEs extraction from the pressed seeds by surfactant solution. The third part is to recovery PEs from the surfactant solution obtained from the pressed seeds extraction step. To fulfill the management scheme, the PEs degradation in the Jatropha pressed seed and Jatropha oil (that are source of PEs) under different storage condition and different sample-pretreatment of the pressed seed materials. The scope flow chart is showed in Figure 1-1 and the detail is showed in Figure 1-2.

Part I: PEs solubilization

The solubilization of PEs was the first step for surfactant selection. The effects of structure of surfactant both hydrophilic ethylene number and hydrophobic carbon-chain length and HLB were studied. The aqueous mixture of some surfactants, electrolyte, and co-solvent was tested to find a suitable surfactant composition that produce high PEs solubilization and used in the next part.

Part II: PEs extraction

The selected solutions were studied for extraction of PEs from the pressed seeds. The effect of solid-liquid ratio, surfactant concentration, extraction speed and time were investigated. The nutrition of meal was studied only for crude protein.

Part III: PEs recovery

The separation of PEs from used surfactant solution in part 2 was studied by temperature altering, liquid-liquid extraction, and electrolyte adding. The suitable condition was investigated.

Part IV: PEs degradation

The degradation of PEs in the oil and the pressed seed with the different sample-pretreatment was investigated as the samples are storage under different condition.

1.5. Expected Results

1) Another alternative approach for PEs extraction by surfactant solution that is comparable PEs to the extrction by methanol with the same physical condition.
2) Phorbol esters contain in treated pressed seed is low as non-toxic Jatropha variety.

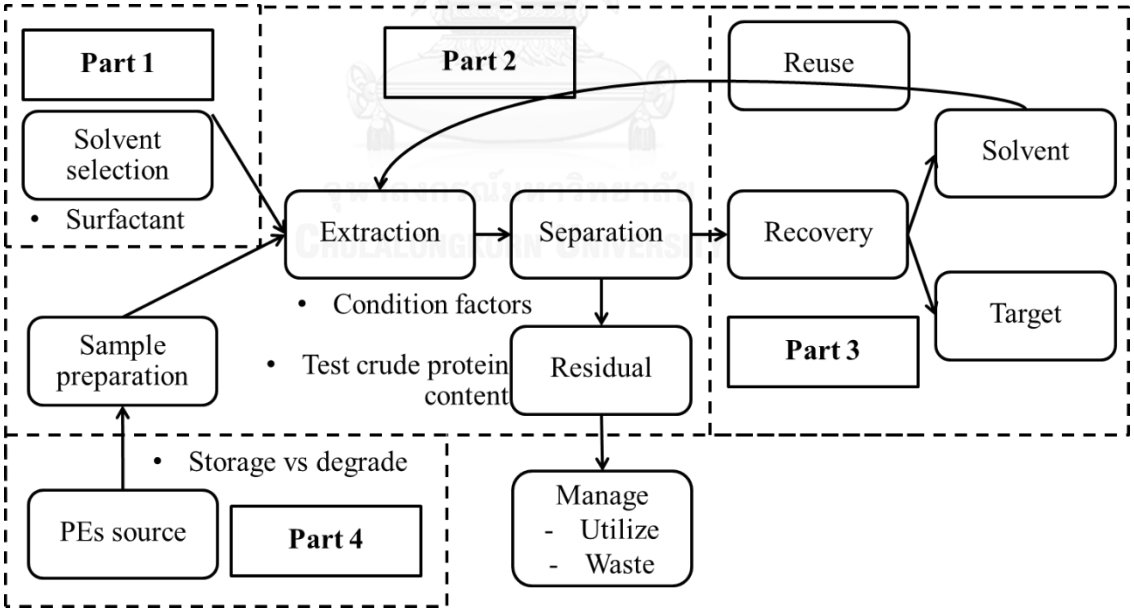

Figure 1-1 Scope of overall research

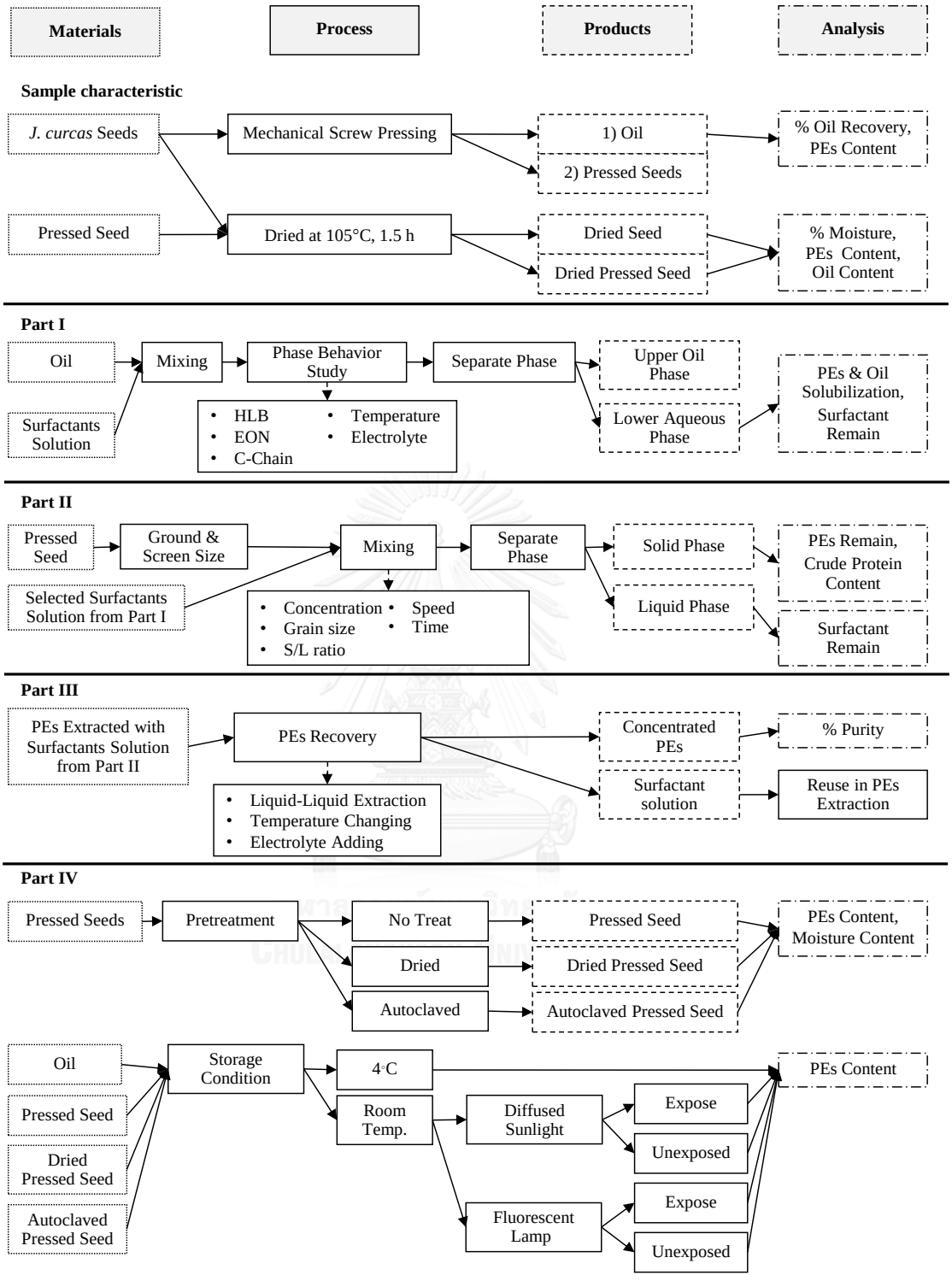

Figure 1-2 Flow chart of the study

CHAPTER II

THEORITICAL BACKGOURNDS AND LITERATURE REVIEW

2.1. Theoretical Background

2.1.1. Phorbol esters

Phorbol esters (PEs) are widely recognized as toxic compounds present in *Croton* sp., *Jatropha* sp., and plants in the Euphorbiaceae family [2, 43]. These compounds are derivatives of the tigliane family of a tetracyclic diterpene, the skeleton structure of PEs group are shown in Figure 2-1a. Several research studies have revealed that PEs are tumor-promoting compounds [44-46] that exhibit toxicological responses in animals that feed on them, even when the PEs are presence at very low concentrations [12, 47-49]. PEs are easily absorbed into the body by the dermal route and the ingestion. The possible effects of contact with PEs are the severe irritation of tissues (the skin, eyes, mucous membrane, and lungs) and induced sensitivity. The first known of PEs is *12-o-tetradecanoyl-phorbol-13-acetate* (TPA) from *Croton* plant and TPA was generally uses as the external standard to determine the concentration of PEs by HPLC [9, 50]. The molecular weight of TPA is 616.92 g mol^{-1} and the formula is $C_{36}H_{56}O_8$. The structure of TPA is showed in Figure 2-1b.

Many studies have reported the concentrations of PEs in *J. curcas* from several sources (Table 2-1). The varieties of PEs detected in *J. curcas* seeds are named as jatropha factors C1, C2, C3, C4, C5, and C6 (Figure 2-1c) [1, 45, 51, 52]. Since PEs are organic compound that able to miscible and/or partition in oil. They are found in both extracted oil and residue meal or pressed seeds of Jatropha oil recovery process. PEs cannot be removed easily by heat treatment [12]. Even though these compounds have negative effect, there are some potential benefit as bio-pesticide and medicine as show in the literature review part.

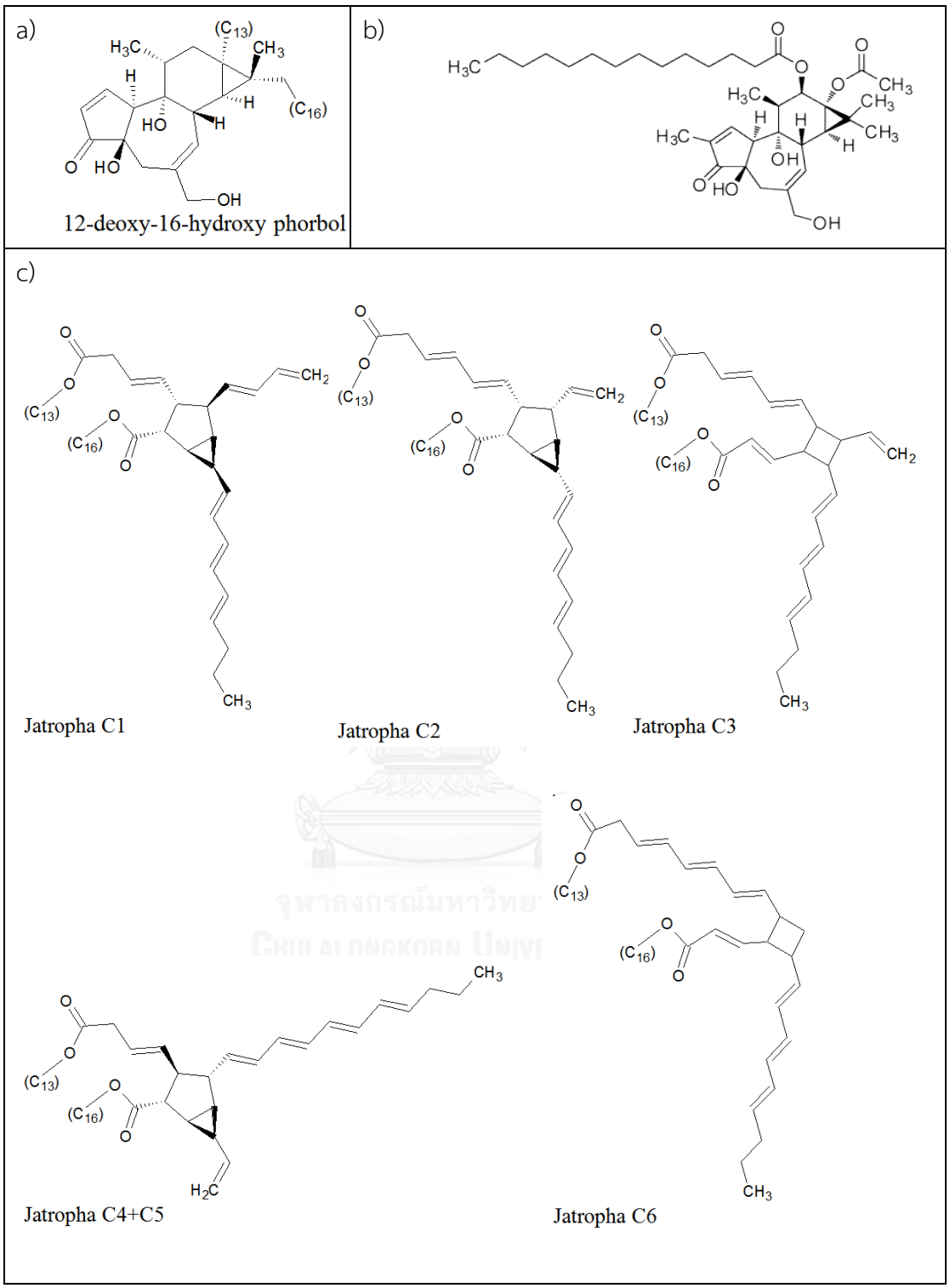

Figure 2-1 The structure of phorbol esters: (a) general structure, (b) 12-o-tetradecanoyl -phorbol-13-acetate (TPA) phorbol esters in Croton seed, (c) phorbol esters in *J. curcas* seed

Table 2-1 Phorbol esters in *Jatropha curcas*

Oil (mg as TPA equivalent/g)	Kernel (mg as TPA equivalent g^{-1})
-	2.17-2.70 [21]
-	0.11 (non-toxic Mexico variety) [21]
3.1 [29]	-
3-6 [4]	1-2 [4]

2.1.2. *Jatropha curcas* L.

J. curcas is a native plant of Central and South American countries in the Euphobiaceae family [2, 43]. The wide variations of this plant in the morphological characteristics are present on its stems, leaves, flowers, fruits, and seeds. *J. curcas* is a drought-resistant species and widely cultivated in the tropics as a living fence. This plant has been used for many purposes; such as medicine, pesticide, soap production, diesel fuel, etc. [43]. Large amount of oil contained in the seed. This oil could be used directly or indirectly in engines. Besides, kernel meal is one of interest since it contains high nutrient. Chivandi *et al.* [53] reveals that *J. curcas* meals contain crude protein even higher than soybean, for this reason, it has potential to be a superior raw material for animal feed production. Therefore, the develop technique which able to eliminate phorbol ester from seed meal would be a preferred solution.

2.1.2.1. Oil content

J. curcas trends to be more interesting as an alternative fuel because its seed contains the high amounts of oil. Previous studies exhibited the amounts of containing oil in the whole seeds and in the kernels (Table 2-2). The most oils of *J. curcas* are in triglyceride form [2]. The compositions of free fatty acid exist in the oil is showed in Table 2-3.

2.1.2.2. Nutrients in seeds

The *J. curcas* seed meal is rich with nutrient. Crude protein content of 26% was observed [50]. The content of crude protein in *J. curcas* kernel is higher than that of soybean [53]. There are many studies reveal the amount of crude protein found in each parts of *J. curcas* seed (Table 2-4). The essential amino acids of meals almost

meet the FAO reference protein [54]. However, the kernel is not suitable for animal feeds as it contains some toxin as well.

Table 2-2 Oil proportion in *Jatropha curcas* seeds by percent weight

Whole seed	Kernel
-	48.5 [57]
37.4 [55]	46-48.6 [55]
-	58-60[58]
30-50 [56]	45-60 [56]

Table 2-3 Free fatty acid in *Jatropha curcas* oil

Fatty acid		Formula	Systemic name	%wt.
Palmitic	C16:0	$C_{16}H_{32}O_2$	Hexadecanoic	13.38-19.5
Palmitoleic	C16:1	$C_{16}H_{30}O_2$	cis-9-Hexadecanoic	0.88-0.9
Stearic	C18:0	$C_{18}H_{36}O_2$	Octadecanoic	2.3-7.4
Oleic	C18:1	$C_{18}H_{34}O_2$	cis-9-Octadecanoic	34.3-49.0
Linoleic	C18:2	$C_{18}H_{32}O_2$	cis-9,cis-12-Octadecanoic	29.7-43.2
Total saturated				20.8-26.3
Total unsaturated				72.7-78.7

Note: Adapted from [55, 57, 59, 60]

Table 2-4 Crude protein content in Jatropha curcas seeds by percent weight

Seed	Kernel	Meal*
26.75 [61]	-	-
-	22.2-27.7 [54]	57.3-64.4 [54]
24.60 [59]	-	-
-	-	57.7 [53]

Note: * Meal is defatted kernel

2.1.3. Oil Extraction Method

The general method to extract oil from the *J. curcas* seeds is pressing technology. This technology is used in small and medium scale and there are some different in the machine characters; nevertheless, this method cannot extract all the oil from the seed. Therefore, the organic solvent has been used in large scale or industrial extractions to rise up the extractable oil [2]. The efficiency of different oil extraction technologies are showed in Table 2-5.

Table 2-5 The Efficiency of oil extraction [2]

Method	Efficiency (%)
Pressing technology	
- Hand powered small scale pressing	60
- Mechanized pressing equipment	75
- Commercially available pressing system	90
Industrial extraction with organic solvent (mainly hexane)	nearly 100

2.1.4. Phorbol esters Removal Method

Many researchers studied about the method to remove PEs from the Jatropha pressed seeds for feedstock aspect. Most of the previous research on PEs removal from *J. curcas* oil and seeds has focused on organic solvents [13, 14], strong base solutions and adsorbents [11]. A high-efficiency PEs removal approach is PEs destruction with base followed by washing with alcohol [10, 11]. The summary method is shown in Table 2-6 and Table 2-7 for the pressed seeds and oil, respectively.

Table 2-6 Phorbol esters removal method for Jatropha pressed seeds

Solution	Removal method				% Eff.
	Ratio S:L w/v	Equipment	Temp. (°C)	Time (min)	
2% KOH + 95% Ethanol	1:5	Stirrer	30	45	70 – 78 [11]
				One night	<0.11 mg/g [11]
90% Ethanol + 0.07% NaHCO3	1:10	Stirrer	Room	120	97.9 [10]
66% moisture	1:2	Autoclave	121	30	0 [12]
4×92% methanol	1:2	Autoclave	121	30	94.9 [12]
4% NaOH +10% NaOCl	1:2	Autoclave	121	30	92.7 [12]
4% NaOH + 2×92% methanol	1:2	Autoclave	121	30	ND [12]
4% NaOH + 4× dist. water	1:2	Autoclave	121	30	ND [12]

Table 2-7 Phorbol esters removal method for Jatropha oil

Solution	Removal method					% Eff.
	Ratio Oil:Sol. w/v	Equipment	Speed (rpm)	Temp. (°C)	Time (min)	
Bentonite 200	3.2% Abs:Oil	Stirrer	100	room	15	98.7 [11]
Ethanol	1:1	Magnetic stirrer	300	23	15	52 [14]
2% Dichloromethane in methanol	1:1	Magnetic stirrer	300	23	15	83.5 [14]
2% Tetra hydro furan in methanol	1:1	Magnetic stirrer	300	23	15	83 [14]
2% 1:1 DCM:THF (v/v) in methanol	1:1	Magnetic stirrer	300	23	15	87 [14]
Methanol	1:2	Magnetic stirrer	300	23	5	77.7 [13]
	1:2	High shear mixer	13,000	23	2	80 [13]

2.1.5. Surfactants

"A surfactant (a contraction of the term surface-active agent) is a substance that, when present at low concentration in a system, has the property of adsorbing onto the surfaces or interfaces of the system and of altering to a marked degree the surface or interfacial free energies of those surfaces (or interfaces). The term interface indicates a boundary between any two immiscible phases; the term surface denotes an interface where one phase is a gas, usually air" [20]. Surfactant structure is amphipathic; in the other word, one surfactant molecule consists of both hydrophobic group and hydrophilic group. Surfactants are classified into 4 types by ionic head group: anionic, cationic, zwitterionic, and nonionic.

2.1.5.1. Anionic Surfactants

The hydrophilic group of this surfactant type was a negative charge. It is sensitive with ionic strength, while less sensitive with temperature. Most anionic surfactants are excellent water soluble and produce low viscosity [20, 62]. The optimal hydrophobic chain for detergency is linear alkyl chain of 12 – 16 carbons. Linear carbon chains are more degradable and more effective than branched chain. [62] Sodium dodecyl sulfate (SDS), which is linear alcohol sulfate group, is a good detergents in the low hardness water [20]. The function of ethylene oxide (EO) in anionic surfactant molecule can reduce the ionic sensitivity [20] such as SDS extended with 3-EO called sodium lauryl ether sulfate (SLES) [62].

2.1.5.2. Nonionic Surfactants

There is no ionic charge in this type of surfactant molecule making it is generally insensitive with ionic strength in the solution [20, 62]. Thus, nonionic surfactants are more hardness tolerant than ionic surfactants. Moreover, they are not caused protein denature. [20] However, nonionic surfactants are temperature sensitive; they can be separated from the water under high temperature depended on the structure of them and some additives in solution [20, 62]. The viscosity of nonionic surfactant solution is rapidly increased with the concentration and temperature rising, especially the nonionic surfactants with higher EO [62]. Polysorbate (Tween®) and Sorbitan (Span®) are food additives. Generally, polysorbate contains 20 EO in molecule and is water-

soluble; while, sorbitan is no EO and water-insoluble. [62] Fatty alcohol ethylene oxide (AE) group can be adjusted from water-soluble to water-insoluble by EO number in molecule [62]. AEs are biodegradable and are excellent detergents for oil removal from oily soil [20].

2.1.5.3. Hydrophile-Lipophile Balance (HLB)

HLB value demonstrates the hydrophilic and hydrophobic portions in molecule of surfactant, and indicates the behavior of each surfactant. HLB is derived from the calculation that range from 0 – 40. [20] A higher HLB of surfactant is a trend to emulsify in more polar phase, while a lower HLB surfactant likes to emulsify with non-polar phase. The HLB of mixed surfactant solution is calculated by equation 2-1.

$$HLB = \sum(HLB_n \times X_n) \qquad \text{Equation 2-1}$$

Where, HLB_n is HLB of the surfactant and X_n is weight fraction of the surfactant in system.

2.1.5.4. Interfacial Tension Reduction Phenomena

In liquid phase, the energy to bring one molecule at interior phase is smaller than to bring it at surface or another phase. The minimum energy that used to bring molecule from interior to surface is called surface tension or interfacial tension (IFT). IFT is surface free energy per unit area. The two bulk phases have potential energy between phases greater than the same bulk phase or interior phase. IFT ($\gamma_{i)}$ of two different phases is the combination of the surface free energies per unit area of each phase minus with the interaction per unit area across the interface as expressed equation 2-2.

$$\gamma_i = \gamma_a + \gamma_b - 2\gamma_{ab} \qquad \text{Equation 2-2}$$

The surface free energies per unit area of two phases are γ_a and γ_b, and γ_{ab} is the interaction energy per unit area across between two phases. Surfactants can reduce the IFT between immiscible phases (i.e. water/oil) by their amphipathic structure. The hydrophilic part is able to adsorb with the polar phase and the hydrophobic part is able to adsorb with the non-polar phase. Thus, surfactants are lined in the between phases and caused the reduction of tension across the interface

[20]. Moreover, the IFT is decreased when concentration of surfactant is increased until the surfactant concentration reaches the critical micelle concentration [62].

2.1.5.5. Solubilization

The definition of solubilization is “the spontaneous dissolving of a substance (solid, liquid, or gas) by reversible interaction with the micelles of a surfactant in a solvent to form a thermodynamically stable isotopic solution with reduced thermodynamic activity of the solubilized material” [20]. At concentrations above the critical micelle concentration (CMC), surfactants can form micelles and cause less polar or nonpolar compounds to solubilize within the micelle [20]. In micelle, the solubilization of compounds can be occurred at any location of micelle depending on the properties and structure of solubilizate and surfactant. The locus of solubilization normally categorized for the normal micelle or oil in water as demonstrate in Figure 2-2. Non-polar solubilizate mainly located in the core of micelle, while polar solubilizate likely located near the shell or surface of micelle. [20]

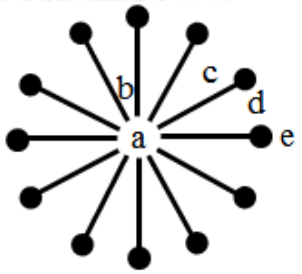

Figure 2-2 Locus of solubilization in aqueous surfactant solution: a) in inner core, b) in deeper palisade layer, c) in outer palisade layer, d) between hydrophilic group, and e) on surface of micelle.

Many researchers have observed that the solubilization of low-water-soluble organic compounds can be enhanced in water in the presence of a nonionic surfactant [21-24]. An increase of the hydrophilic moiety in surfactant molecules with the same hydrophobic moiety can increase the solubilization of polar compounds; in contrast, nonpolar compound solubilization is dependent on the hydrophobic component of the surfactant [20, 21].

In case of nonionic surfactants system, surfactants are generally more hydrophobic at high temperatures [63]. Dehydration between the EON of a micelle

(palisade) occurred when the temperature increase resulted in a closely packed palisade region. Increases in the aggregation number of micelles and expansion of the micelle size result in a larger inner core for nonpolar compound solubilization [20]. Consequently, the solubilization of polar compounds in micellar solution decreased [20].

In case of anionic surfactants system, the addition of electrolyte reduces the electrorepulsive force between the head portion of the monomer in surfactant micelles, resulting in a reduction of the CMC and an increase of the aggregate number of micelles [20, 63]. With an increase in the greater aggregate number of a micelle, the solubilization of an organic solubilizate would be increased.

Moreover, mixing anionic and nonionic surfactants can prevent nonionic adsorption and partitioning into an organic oil [25], and the resulting mixture has a synergistic effect on solubilization enhancement [26-28]. The factors that enhance polar compounds and non-polar compounds in normal surfactant-micelle system are summarized in Table 2-8.

Table 2-8 Solubilization enhancement factors

Surfactant type	Nonpolar compound	Polar compound
Nonionic	Increase hydrophobic part i.e. carbon chain length Long chain alcohol adding Increase temperature	Increase hydrophilic part i.e. ethylene oxide number Short chain alcohol adding Decrease temperature
Anionic	Electrolyte adding	Increase anionic head group

Source: Rosen [20]

2.1.5.6. Cloud point phenomenon

Theoretically, solution of polyethylene oxide nonionic surfactant will be separated in two phases known as surfactant-rich phase and dilution phase at above a certain temperature. This phenomenon is called “cloud point”. As a result of phase separation, solubilizates or extracted target will also be concentrated in the rich phase [20, 33]. Some electrolytes are able to dehydrate water from the EO head group of surfactant that decrease the CP, called salting out effect [20]. In addition, the low

hydrophile-lipophile balance (HLB) surfactant can adjusted the cloud point temperate [34-36]. The factors that related with cloud point are summarized in Table 2-9.

Table 2-9 Factor effecting on cloud point temperature of nonionic surfactant system

Factors	Cloud point temperature	
	Increase	Decrease
Structure of surfactant	Ethylene oxide number	Carbon chain length
Additive	Salting in electrolytes	Salting out electrolytes
	Higher HLB surfactants	Lower HLB surfactants
	High polar compounds	Less polar compounds

Source: Rosen [20]

2.1.6. Extraction Process

The extraction process normally summarized the main step as in Figure 2-3. The factors that affect the extraction efficiency include sample properties, solvent properties, and extraction condition. Sample preparation is necessary process to extract the target compounds from the solid sample[30]. The reduction of particle size is necessary to increase the mass transport from sample to extract solvent. [31] The solvent need to high efficiency for separating the targets from the matrix and is stable to the target [31, 32]. The physical condition play important role in the extraction process related mass transfer such as solid to liquid ratio, temperature, mixing type, mixing speed, and contact time [31, 32]. The factors related with extraction process are summarized in Table 2-10.

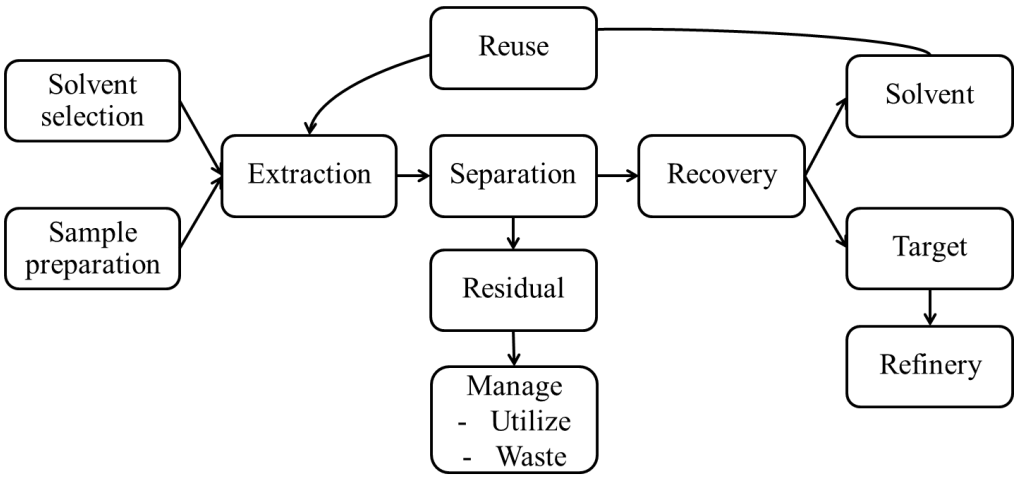

Figure 2-3 Solid phase extraction process schemes

Table 2-10 Criteria factors for extraction process

Criteria	
Materials preparation [64]	- Size reduction: increase surface area on solvent contacting with samples - Moisture: depend on the solvent properties, mostly should be 2-6%
Solvent selection [30, 65]	- Capacity: high concentration of solute loading - Selectivity: selective for the desired solute over impurities - Solvency: minimal solubility in the raffinate phase - Reversibility: reversible and recovery to extraction process - Availability: available more than one supplier - Physical properties: optimal interfacial tension, low viscosity - Cost & Safety: inexpensive, nontoxic, noncorrosive
Systems of mixing [66]	In this study, solid-liquid extraction - Mixing equipment: appropriate for purpose - Dimensions - Geometrical shape - Velocity of agitation: off-bottom precipitation of samples. - Time of mixing

2.2. Literature Review

2.2.1. Phorbol esters Observation

As generally being recognized, *J. curcas* seeds not only contain oil with high nutrient but also contain several types of toxin. Adolf *et al.* reports that 12-deoxy-16-hydroxyphorbol extracted from *J. curcas* oil by methanol irritate to the ear of rat [44]. Makkar *et al.* [50] extensively studied on the nutrient and toxin of different provenances of *J. curcas*. Eighteen *J. curcas* in different planting areas from the West and East Africa, North and Central America, and Asia were used in this experiment. They found that there is the diversity in the amount of nutrient and toxin in variety of seed stains. The kernels contain crude protein (19 – 31) %, lipid (43 – 59) %, neutral detergent fiber (3.5 – 6.1) %, and ash (3.4 – 5.0) %. The toxins in defatted kernels or meals are trypsin inhibitor activity (18.4 – 27.5) mg of trypsin inhibited/g, saponins (1.8 – 3.4) % as diosgenin equivalent, and phytate (6.2 – 10.1) % as phytic acid equivalent. Moreover, PEs remain in 17 provenances around (0.87 – 3.32) mg/g of kernel but cannot be detected in the seeds from Papantla, Mexico. Makkar *et al.* [54] continued their research and found that about 90% of CP in *J. curcas* meals is true protein and the levels of essential amino acid meet the FAO reference protein, except lysine.

In 2005, Chivandi *et al.* [53] compared the nutrient and antinutrient between industrially processed Zimbabwean *J. curcas* and *Glycine max* (soybean) meals. The soybean meals were derived by hexane extraction and the Jatropha meals were derived by hexane-ethanol extraction. They found that Jatropha meals contain CP 577.00 g/kg dry mass that has a significant *($p<0.05$)* higher than 470.80 g/kg dry mass of soybean. However, PEs were also found in the Jatropha meals about 0.8 mg/g while it cannot be observed this compound in soybean meals. Further result shows that the hexane-ethanol extraction can reduce PEs from 6.5 mg/g of raw kernels to 0.8 mg/g, 87.69% PEs reduction.

2.2.2. Phorbol esters Potential Utilization

Although PEs are toxic compound, they are considered as a useful compound. PEs were found to have potential for agricultural as pesticide [3-5], and pharmaceutical purpose as be used for leukemia remedy [6] and as the initial substance for prostratin synthesis for HIV treatment [7].

Rug and Ruppel [3] found that the methanol extract from *J. curcas* oil for enrich PEs, the aqueous extract and *J. curcas* crude oil toxify to intermediate snail hosts and larvae of schistosomes. Among these three liquid, the methanol extract has the highest toxicity to the snails at LC_{50} = 5 ppm and LC_{100} = 25 ppm while Bayluscide® which is a commercial pesticide for snail killing can kill all snails at 1 ppm. Verma *et al.* [4] investigated that PEs fraction from *J. curcas* oil is an effective bio-pesticide to control the *Odontotermes obesus* termites. All test termites died after 0.5 g/mL PEs exposure within 12 h. LC_{50} of PEs to the termites is 0.071 g/mL at 24 h. Toxicity of PEs may not be compatible with the commercial chemical pesticide; however, PEs can be considered as bio-product. In corn field, *Spodoptera frugiperda* is an important pest, Davappa *et al.* [5] selected PEs fraction from *J. curcas* oil to test the toxicity with the third instar larvae of this pest. They found that LC_{50} of this compound to the larvae is at 0.83 mg/mL treated on corn leave. At lower PEs concentrated treated (0.0625 – 0.25 mg/mL), the mortality was not found; however, the adverse effect occurred on reduction of food consumption, growth rate, and food conversion efficiency.

In addition, PEs is possible to use for treatment leukemia. In 1990, Scher *et al*. [67] studied the effect of PEs with the secrete and white-blood-cell of the patients with myeloid leukemia and found some positive effect of PEs in the study. Moreover, Jones *et al.* found that PEs has positive effect in anti-leukemic activity in vitro. In 1996, Mihalik *et al.* [68] observed that PEs was able to inhibit human lymphoblastic cell growth. In addition, Chang *et al*. [6] claim that PEs and particularly TPA has an "effective in treating patients with neoplastic diseases such as leukemia as well as in increasing the white blood cell count". Devappa *et al.* [7] found that PEs can be a primary substance in synthesized prostratin, which is known as antivirus agent.

2.2.3. Phorbol esters Extraction from Jatropha Pressed Seeds

In generally, the jatropha pressed seeds that extracted with methanol contain PEs as lower as a non-toxic jatropha variety [12]. Aregheore *et al.* [12] investigated The best method that can reduce PEs from 1.78 mg/g to 0.09 mg/g is heat-treated and washed 4 times with 92% methanol. This method is better to detoxifying *J. curcas* meal but may be unsuitable in economic term. However, the methanol extraction process requires a special control system because methanol is a highly volatile organic compound that poses an explosion risk. Thus, the reduction of methanol amount in PEs extraction process was needed.

The mixing of other solvents with methanol was investigated. Severa *et al*. [69] used a ionic liquid as co-solvent to reduce the amount of methanol. However, the increasing of co-solvent upper than 60% wt causes the PEs extraction efficiency dramatic decrease due to the increase of viscosity of the solvent. Their optimal extraction condition is 30% wt of ionic liquid per 70% of methanol, solid to liquid ratio of 1:16.5 (w/w), and 22 h of extract time. Almost 98% of PEs was extracted from the jatropha seeds. Guedes *et al*. [15] used ethanol:methanol blending at 50:50 ratio to extract PEs from JPS. The extraction efficiency reached 97.3%, while the optimal extraction condition was 8 h extract time and solid to liquid ratio of 1:10 (w/v).

An alternative solvent, which more environmental safe and non-volatile, was introduced. Phasukarratchai *et al.* [14] found that the extraction efficiency of PEs from Jatropha pressed seeds has significantly related with hydrophile-lipophile balance value of single nonionic surfactant. Anionic surfactant (Aerosol OT) mixing with nonionic surfactant (Tween®80 and Dehydol®LS9) cannot enhance the efficiency. Only 15 min need in the extraction step. The residual pressed seed still contain PEs as low as found in a nontoxic variety. [8, 19]

2.2.4. Phorbol esters Degradation

Several research studies on toxicity and degradation of PEs in environment. A study of the environmental stability and degradation characteristics of PEs by Schmidt and Hecker [37] revealed that 12-o-tetradecanol-phorbol-13-acetate (TPA) is degraded

rapidly at room temperature especially when expose to diffused sunlight, with the powder form of TPA degrading faster than TPA dissolved in solvents. Nonetheless, the degradation was tardy at low temperature as 4°C and halt at -20°C.

Moreover, these compounds can be degraded under sunlight exposure. Yunping *et al*. [38] found that the PEs in Jatropha oil were rapidly degraded to near 100% within nine days under sunlight (80,000 lux of maximum light intensity) at room temperature of approximately 25°C.

Devappa *et al.* [39] who found that the PEs degradation rate in soil mixed with Jatropha pressed seed or with PEs-silica were high and complete within 12 – 23 days depending on the temperature and the moisture content while the PEs degradation of autoclaved samples was not occurred under the same incubation condition. The result indicated that PEs degradation was arisen by the microbes in the soil under the aerobic condition.

The bacterial fermentation can also reduce the amount of PEs in the Jatropha pressed seed. Accordingly, Joshi *et al.* [40] found that *Pseudomonas aeruginosa* PseA can degraded PEs in Jatropha pressed seed under the solid state fermentation to non-detectable level within nine days. Moreover, Phengnuam and Suntornsuk [41] found that PEs reduction occurs under the fermentation by *Bacillus* sp that related with many enzymes. Not only bacteria can degrade these compounds but also fungi. According to de Barros *et al.* [42], PEs can be reduced from the pressed seeds by white rot fungi under the control incubation condition within 30 days to reach as non-toxic level. Moreover, da Luz *et al.* [70] found that *Pleurotus astreatus* mushroom growing on the Jatropha pressed seed can reduced PEs concentration both in the pressed seed and in the mushroom until lower than the non-toxic Jatropha variety.

2.2.5. Extent of Solubilization

The solubilization can be extended depending on the properties of solubilizates and surfactants. In case of nonionic surfactants, the extent of solubilization was studied about the effect of surfactant structure. The solubilization of Alachor (a slightly water-soluble molecule) in nonionic surfactant series with

different EON, Neodol and Triton, exhibited results similar to those of this work in that greater EON values lead to greater solubilization [24]. However, Jafvert et al. have reported that, for a nonpolar organic compound, an increase of the EON of a nonionic surfactant with the same C-chain length decreased the solubilization of hexachlorobenzene [21]. Similar result is revealed by Alam et al. that the reduction of oil solubility occurred with an increase EON of nonionic surfactants [71].

In case of anionic surfactant, the environmental adjustment was investigated. As reported by Damrongsiri *et al.* [72], the addition of an electrolyte to an anionic surfactant can enhance the nonpolar compound solubilization in the micelle core and decrease the solubilization of polar compounds in the palisade layer of the micelles. Ranganathan *et al.* [73] observed that heptane, which is a saturated hydrocarbon, increases the aggregate number of the micelles; i.e., a single micelle contains more surfactant monomer. However, the hydration of the area between the hydrophilic head group of the surfactant is constant. Heptane expands the size of the micelles without inducing a packing effect on the head group of the surfactant; however, when NaCl is added to the SDS solution, the micelle shape changes from spherical to rod-shaped micelles at a 4:1 NaCl:SDS mole ratio [73].

2.2.6. Locus of Solubilization

The locus of solubilization in normal micellar system is classified as shown in Figure 2-2. Other researchers have previously investigated the locus of organic compounds that exhibit some functional group bonding in structures similar to PEs. For example, polyaromatic hydrocarbons (PAHs), benzene, and nitrotoluene contain unsaturated bonding in cyclic structures, and phenol contains a hydroxyl group. Kandori *et al.* [74] observed similar results with phenol solubilized within the ethylene oxide (EO) palisade of polyethoxylated nonylphenols. Similarly, Parekh *et al*. [75] reported that phenol was located at the EO of Tetronic 904 (a tetrafunctional block copolymer based on EO and propylene oxide (PO) nonionic surfactant) micelles, whereas benzene was located deeper in PO in the palisade region. In addition, PO is more hydrophobic than EO. Luning-Prak *et al.* [76] demonstrated that nitrotoluene

solubilized into the shell and then into the core of nonionic micelles (polyethylene oxide linear alkyl ether (Brij®), polyethylene oxide octylphenyl ether (Triton®), and polyethylene oxide alkylphenyl ether (Tergitol® series) when the nitrotoluene concentration was increased. In the case of PAHs, Bernardez [77] demonstrated that PAHs, which are slightly water-soluble, were solubilized into the shell of nonionic surfactants (Brij®35, Brij®30, Tween®80, Triton®X-100 and Tergitol®NP-10) between EO at low PAH concentrations and deep into the core of the micelles at high PAH concentrations. Moreover, Takeuchi *et al.* [23] reported nuclear magnetic resonance spectroscopy results that confirmed that naphthalene was located in the palisade region or EO of heptaoxyethoxylated monohexadecyl ether ($C_{16}EO_7$).

2.2.7. Surfactant for Extraction

In the previous studies, surfactant water-based solutions have been introduced as alternative solvents for organic compounds extraction from the matrix of plants and water. For example, Ribeiro *et al.* [17] found that surfactant extraction and cloud point separation has the comparable efficiency with ethanol solvent. The optimal condition to extract saponins from *Agave sisalana* (sisal) is 7.5% (v/v) of Triton® X-100 and 20 (w/v) of sodium carbonated is needed in cloud point separation process with the highest saponins concentration factor. Sharma *et al.* [78] found that the phenolic contents and antioxidants extraction efficiency from fruit juice are related with HLB of surfactants. Brij®58 shows the highest efficiency than SDS, Brij®35, Triton® X-100 and Span®40. Mixed surfactant systems were found to yield higher effectiveness than that of a single surfactant in some studies. Do *et al.* [79] found that mixture of anionic-cationic-nonionic surfactants has less ultra-low interfacial tension with soybean oil than single nonionic surfactants and had the higher extraction efficiency.

2.2.8. Cloud Point Separation Technique and Application

Cloud point separation is a technique use for the separation of nonionic surfactants from aqueous surfactant solution. The temperature requirement can be designed with structure of surfactant selection and some additive. High

polyoxyethylene (POE) in molecule cause high cloud point [20]. Impurity in the surfactant solution can cause either increasing and decreasing the cloud point temperature. An increasing of electrolytes generally effect on cloud point lowering, Schott *et al.* [80] found that the cations of Na^+, K^+, Cs^+, and NH_4^+ is salting out with no complex formation with POE surfactants. The anion of OH^-, F^-, Cl^-, SO_4^{2-}, and PO_4^{3-} have ability to reduce cloud point temperature, while I^-, $[Fe(CN)_5NO]^{2-}$, and SCN^- salted show opposite results. Moreover, Schott [81] found that the degree of salting-out is depending on the number of POE in nonionic surfactant molecule. In mixed surfactants system, Batıgöç *et al*. [82] found that cationic surfactant mixing with Triton® X-100 show the cloud point increasing when compared with single Triton® X-100.

The applications of cloud point phenomena are used in a wastewater pretreatment [83], a sample preparation and concentration for analysis [84], and an extraction and recovery of target compounds [17, 85]. To demulsify oil from wastewater, Tong *et al.* [83] found that calcium chloride additive is able to separate the emulsion in super heavy oil wastewater in two layers and the COD removal efficiency is more than 90%. Trace contaminants in food are important aspect and required sample preparation to concentrate the analysts before analysis. Liu *et al.* [84] found that the cloud point extraction by Triton® X-100 is effective and reliable procedure for concentrating triazine herbicides from milk sample before analyze by HPLC.

Pan *et al.* [85] use the nonionic surfactants system in lipase production from *Serratia marcesens* ECU1010. They found that the fermentation under Triton® mixture can be occurred. Two phase fermentation by cloud point at 6% of total Trition® X-114: Triton® X-45 (4:1) is no toxic to this microorganism. Almost lipase is partition in surfactant rich phase; however, surfactant concentration is inhibiting lipase activity. Thus, the surfactant separation is required.

For recovery nanoparticles, Nazar *et al.* [33] was applied cloud point extraction to recovery the expensive nanoparticles (NPs) and concluded that NPs recovery by surfactant can use as colloid solution. The efficiency of this method is up to 50%.

CHAPTER III

METHODOLOGY

All results in this study were conducted at the laboratory Chulalongkorn Research Building, floor 10th, Chulalongkorn University. Since *Jatropha curcas* seeds, oil and pressed seeds are natural products, in order to reduce error from variation in composition of the specimen, the same batch of these natural products was used for the whole experiments. Moreover, to ensure reliable results, all experiments were carried out in triplication.

3.1. Materials

3.1.1. *Jatropha curcas* seeds, pressed seeds (or seeds cake) and oil were supplied by Kasetsart University, Kamphaengsaen Campus, Nakhon Pathom, Thailand (WGS84: 14.03403, 99.97032). The seeds were collected in April, 2012 from mature *J. curcas* trees and were squeezed using a screw press to separate the oil. The oil was stored in opaque, sealed glass bottles and the pressed seeds were stored in opaque, sealed plastic bag, in order to protect from light, and were kept at 4°C until it was used for the experiment.

3.1.2. Solvent: methanol and acetonitrile (HPLC grade), ethanol (Analytical grade), and de-ionized water 18 MΩ•cm

3.1.3. 12-o-tetradecanoyl-phorbol-13-acetate [TPA] was used as a PE standard for the HPLC analysis. It was purchased from Sigma (100% purity, Lot# BCBF6633V).

3.1.4. Electrolyte: Sodium chloride (NaCl), Calcium chloride ($CaCl_2$), Magnesium chloride ($MgCl_2$), Sodium fluoride (NaF), Potassium chloride (KCl), Sodium nitrate ($NaNO_3$), Sodium carbonate (Na_2CO_3), Sodium sulfate (Na_2SO_4), tri-Sodium phosphate dodecahydrate ($Na_3PO_4 \cdot 12H_2O$) were analytical grade.

3.1.5. Surfactants

3.1.5.1. Nonionic surfactants

1) Fatty alcohol ethylene oxide: the hydrophobic part (fatty alcohol C12-14) was consistent, whereas the ethylene oxide number (EON) varied. This series included laureth-1, laureth-2, laureth-3, laureth-7, laureth-9 and laureth-12, where the number represents the number of ethylene oxide groups in each molecule (Figure 3-1a). All surfactants in this group were of commercial grade and were supplied by Thai Ethoxylate Co., Ltd and the trade name of these surfactants is Dehydol®LS.

2) Sorbitan group (Span): Monolaurate is indicated by 20, monopalmitate is indicated by 40, monostearate by 60 and monooleate by 80. (Figure 3-1b)

3) Polyoxyethylene (20) sorbitan group (polysorbate): each surfactant in this series contained the same head group (hydrophilic polyethylene oxide (20) sorbitan) and a different hydrophobic tail group (C-chain). The polyoxyethylene sorbitan monolaurate, or polysorbate 20 and polyoxyethylene sorbitan monooleate, or polysorbate 80 were purchased from Ajax Finechem. The polyoxyethylene sorbitan monopalmitate, or polysorbate 40 and polyoxyethylene sorbitan monostearate, or polysorbate 60 were purchased from Merck. (Figure 3-1c) The trade name of this series is Tween®.

3.1.5.2. Anionic surfactants.

Anionic surfactants, sodium dodecyl sulfate (SDS, $C_{12}H_{25}SO_4Na$), was purchased from Ajax Finechem (Figure 3-1d) and sodium laureth sulfate (SLES, $CH_3(CH_2)_{11}(OCH_2CH_2)_nOSO_3Na$) was purchased from Cognis (Figure 3-1e).

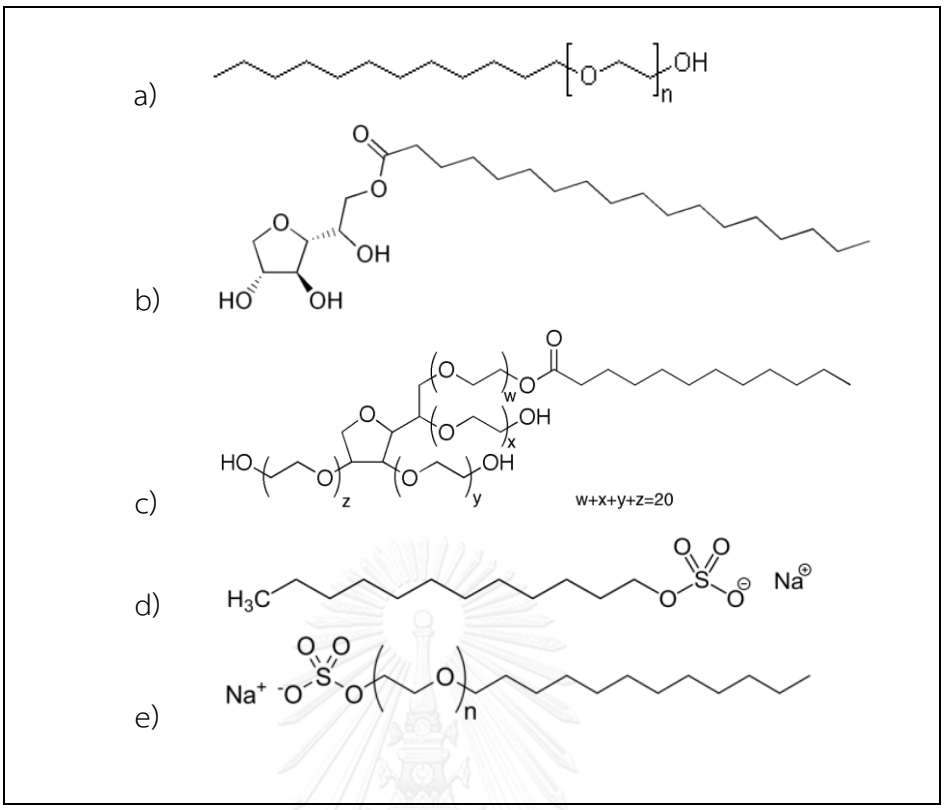

Figure 3-1 Surfactant structure: a) Laureth, b) Span, c) Polysorbitan, d) SDS, and e) SLES

3.2. Methods

3.2.1. Jatropha Seeds Preparation

Prior to the experiment, the average weight of a seed, shell and kernel, and water content were determined by randomly sampling. An average weight of seed was calculated from dividing total weight of seeds by the number of seeds. The seeds were carefully cracked and removed shells then weigh shells and kernels. Shells and kernels were dried in an oven at 105°C for 1.5 h, and then kept in a desiccator to reach room temperature and weighed them again. The kernel: shell ratio was calculated by dividing the average weight of kernel by the average weight of shell. The seed components and the materials are shown in Figure 3-2.

3.2.2. Critical Micellar Concentration (CMC) Determination

The surfactants concentration series were prepared with deionized water. The concentration series of Laureth7, Laureth9, Laureth12, Polysorbate-20 and Polysorbate-40 contain of (0.005, 0.0075, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, and 1) mmol L^{-1}. The concentration series of Polysorbate-80 contain of (0.004, 0.006, 0.008, 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 1) mmol L^{-1}. The concentration series of SDS contain of (0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 50) mmol L^{-1}. For the effect of NaCl on CMC of SDS, the solutions were prepared in the ratio of (0, 0.5 1, 2.5, 5, and 10) mole of NaCl per 1 mole of SDS for each SDS concentration in the series. The CMCs of the surfactants were estimated from the plot of the concentration of the surfactant versus the surface tension at the point where the curve becomes constant (Appendix A, Figure a-1).

Figure 3-2 *Jatropha curcas* seed component

3.2.3. Phorbol esters Solubilization from the Jatropha Oil by Surfactant Solution

The PEs solubilization was evaluated for a single nonionic surfactant, a single anionic surfactant, and mixed surfactant solutions system. The effect of surfactant structure is observed from the comparison of the PEs solubilization by different single

surfactant solutions as shown in Figure 3-3. Because it was not possible to use purified PEs in these experiments, the PEs for the solubilization study were obtained from those present in jatropha crude oil. Thus, 3 mL of surfactant solution was gently mixed with 3 mL of jatropha oil and left at room temperature (or in a controlled-temperature incubator in the case of the experiment where the effect of temperature was investigated) for a week to reach equilibrium. In addition, all samples were kept in a cabinet in order to avoid any light exposure. The aqueous phase was sampled and analyzed for the concentration of PEs, jatropha oil, and surfactant. The total concentration for all surfactant solutions used in the PEs solubilization study was fixed at 20 mmol L^{-1} which higher than CMC of surfactant. The surfactant solutions which exhibited high PEs solubilization with low oil solubilization were selected for the next experiment of the pressed seed.

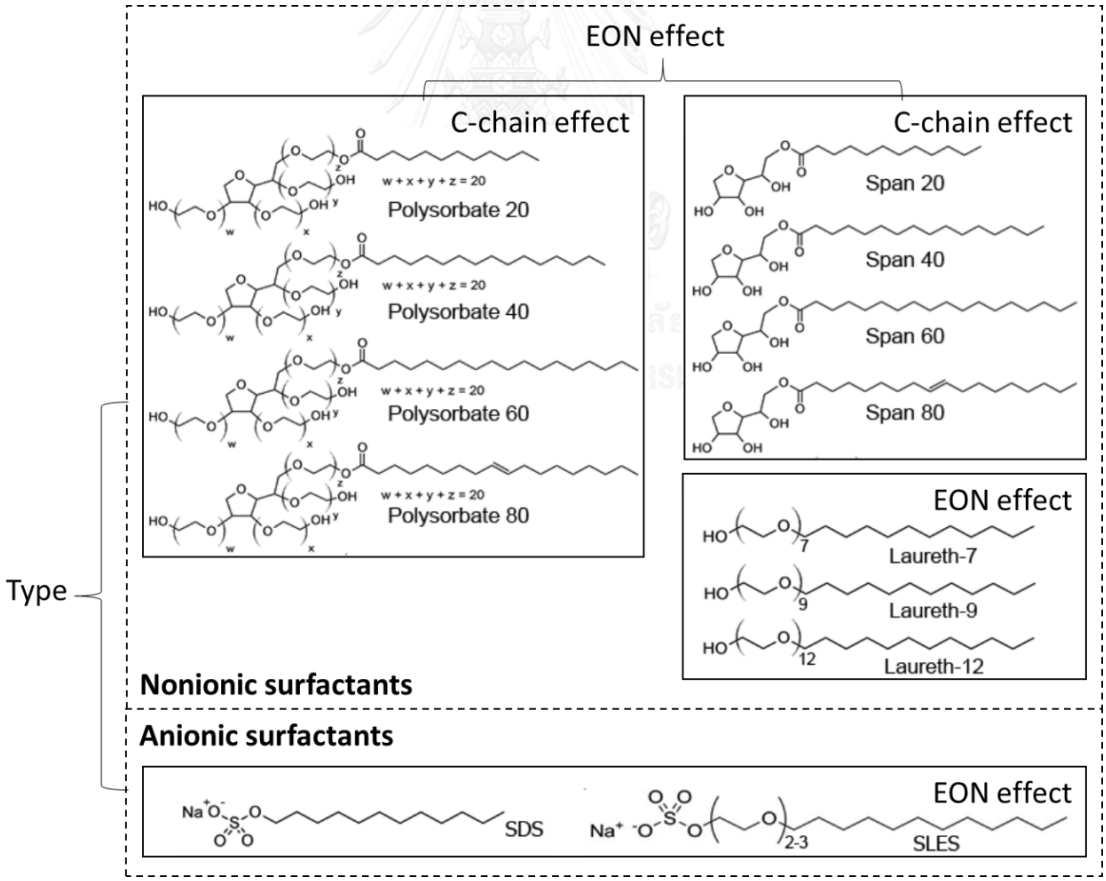

Figure 3-3 Experimental design for effect of surfactant structure on PEs solubilization

3.2.4. Determination of the Molar Solubilization Ratio

The molar solubilization ratios (MSRs) of the PEs were evaluated for each surfactant. The MSR was calculated as the slope of the graph of the concentration of the surfactant minus the CMC and the solubilization above the CMC [22, 86]

3.2.5. Phorbol esters Extraction from the Pressed Seed by Surfactant Solution

3.2.5.1. Surfactant screening

The effect of surfactant type, surfactant concentration, and oil content in JPS were investigated in small scale with an orbital shaker. The PEs in JPS were extracted with fixed physical condition followed in Phasukarratchai *et al.* [19]. The ground JPS were mixed with the surfactant solution at a designed 1:10 of solid to liquid ratio in 40 mL-test tube with screw cap. The samples were mixed by orbital shaker at 300 rpm for 30 min. After that, it was kept for 30 min to settle meals from surfactant aqueous phase then filtered the residual meals. The filtered meals were dried and analyzed for the PEs content and calculated the PEs extraction efficiency from the PEs initial concentration in JPS.

For the effect of oil content, the JCO were extracted with methanol at 1:1 by volume ratio and for four times to get the PEs-free oil. The PEs-free JCO were spiked in the ground JPS in order to adjust the oil content in JPS. The initial oil content in JPS is 16.88 g of crude oil in 100 g of pressed seeds.

3.2.5.2. Extraction condition optimization

The extraction with agitator was designed with fixed geometry for every experimental set in order to avoid an error from different mixing pattern. The geometry design is set for solid-liquid mixing process guideline [87, 88] as top enter agitator with overhead stirrer (DragonLab Model OS20-Pro) with an axial flow turbine. Diameter of tank (D_T) and turbine (D_a) is respectively 10 cm and 5 cm, which D_a/ D_T is 0.5. The turbine was set above the bottom of tank around $D_T/4$ (2.5 cm). The height of mixture (H) is set as H/ D_T range between 0.75 – 1.5 (7.5 – 10 cm). The geometry design and the overhead stirrer used in the experiments are shown in Figure 3-4.

The 550 mL of surfactant solution was fixed for each experimental batch in order to follow the geometry of agitation. The ground JPS were mixed with the surfactant solution at design solid to liquid ratio in 2 L-beaker as following the experimental design. Then the samples were transfer to 750 mL-centrifuge bottom and separated the residual solid and the extract solution by a swinging bucket centrifuge (Thermo Scientific Sorvall 4-Place Swinging Bucket Rotor, 4 x 750 mL) at 2000×g for 15 min. The residual meals were dried and analyzed for the phorbol esters content. The extraction efficiency was calculated based on the initial PEs in the JPS of each batch experiment. The total nitrogen in extracted solutions were analyzed and referred to the extracted crude protein from the pressed seeds.

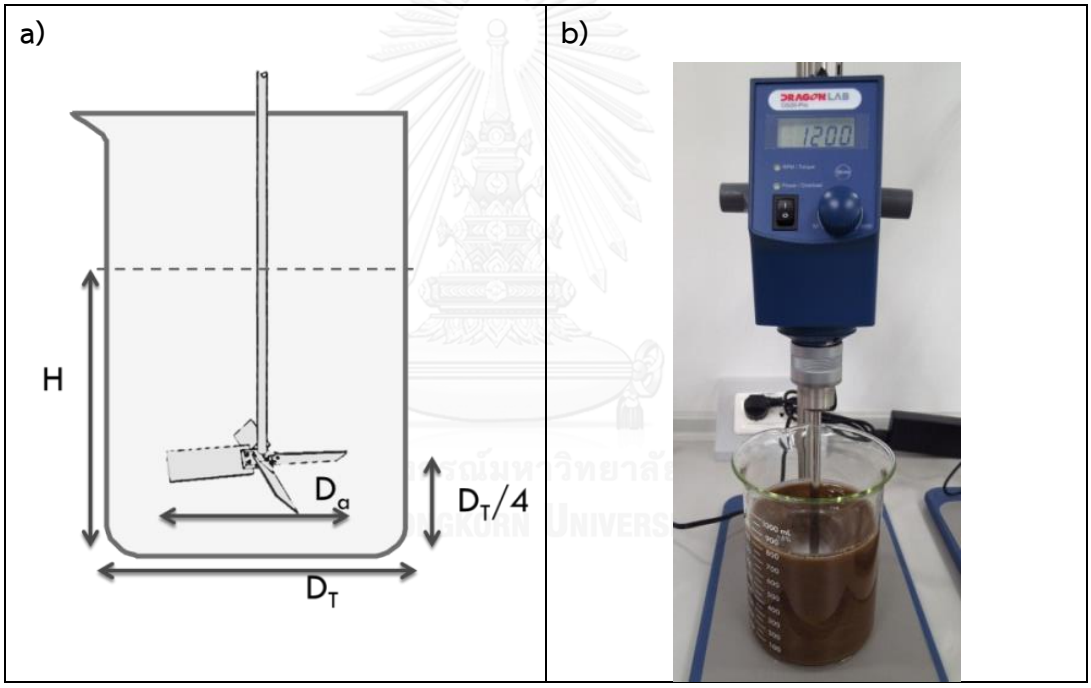

Figure 3-4 Agitation equipment: a) geometry design and b) overhead stirrer

3.2.5.2.1. Factor screening

The extraction factors (surfactant type, concentration, solid-liquid ratio, shaker speed, and time) were screened by using Taguchi robust experimental design in Statistica Program. Each factor was separated in three levels and nine experimental runs were generated with Statistica Program. The interested factors and their level are shown in Table 3-2, and the experimental design set is shown in Table 3-3. The results

were analyzed the significant factors on PEs extraction efficiency with Statistica Program.

Table 3-1 Factors and their levels for Taguchi experimental design

Factor	Level 1	Level 2	Level 3
1. Surfactant concentration (%)	2.5	5	7.5
2. Time (min)	15	30	45
3. Solid to liquid ratio (g: mL)	1:5	1:7.5	1:10
4. Speed (rpm)	400	1000	1600

Table 3-2 Experimental design by Taguchi method for factor screening

Run	Level			
	Factor 1	Factor 2	Factor 3	Factor 4
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

3.2.5.2.1. Optimization of PEs extraction condition

The results from the factor screening by Taguchi method was connected with this step. The speed of agitation was fixed at 1000 rpm and the solid to liquid ratio was fixed at 1 g of pressed seeds per 10 mL of surfactant solution. Only surfactant condition and extraction time were interested. The optimal extraction condition is investigated by using Central composite rotable design (CCRD) in Statistica Program as shown in Table 3-4. The optimal extraction condition was test the ability to reuse the

surfactant solution with fresh JPS. The PEs-surfactant extract solution was used in the PEs recovery process.

Table 3-3 Central composite experimental design for extraction condition optimization

RUN	Factors	
	Concentration (%)	Time (min)
1	2.5	20
2	2.5	60
3	10	20
4	10	60
5	0.9467	40
6	12	40
7	6.25	11.71573
8	6.25	68.3
9 (C)	6.25	40
10 (C)	6.25	40.0

3.2.6. Phorbol estesr Recovery by Cloud Point Separation

The effect of electrolyte on cloud point (CP) lowering was investigated with the pure Dehydol®LS12 or Laureth-12 solution, firstly. The stock solution of electrolytes was added in a 15-mL test tube that contains 2.5 mL of 200 mmol L^{-1} of laureth-12 solution. The total volumes were adjusted to 10 mL and were gently mixed. The mixtures were heating at 0.5°C temperature rising step in a water bath and observed the turbidity then phase separation occurred were recorded.

Some electrolytes were selected to investigate the PEs recovery efficiency with 9.4% (135 mmol L^{-1}) of laureth-12 solution from PEs extraction process or PEs-extract. The solid electrolytes were directly added in 10 mL of PEs-extract, gently mixed, then heated until cloud phase appear. The temperature was increased higher than CP in 5°C and kept constant for 5 minute before sampling the surfactant rich-phase and the

surfactant dilute-phase. The amount of PEs and surfactant were analyzed by HPLC and calculated the recovery efficiencies in rich-phase and dilute-phase; moreover, the amount of electrolyte was analyzed by IC, and then calculated the water amount.

3.2.7. Phorbol esters Degradation under Different Storage Condition

The prepared pressed seeds were pretreated with three different approaches: 1) no treatment, 2) drying in a hot air oven at 80°C for 2.5 h (dried), and sterilization in an autoclave at 121°C for 20 min and then drying, in the same manner as the dried sample (autoclaved). Ten grams of the pressed sample were kept separately for each replicate in a sterilized Pyrex glass petri dish with cover, and sealed with parafilm. The oil in this experiment was used without any treatment and 40 mL of crude oil kept in 40 mL clear I-Chem glass tubes with PTFE screw caps, with each sample in triplicate. In order to simulate general storage condition in housing at room temperature without humidity control and variable protection from light, the oil and pressed seeds in glass containers were stored under the following five different conditions.

(a) *Non-light exposure at 4°C (NL-4C):* The samples stored in glass containers were wrapped with aluminum foil in order to prevent exposure to light, and kept at 4°C in a refrigerator.

(b) *Non-light exposure at room temperature (NL-RT):* The samples stored in glass containers were wrapped with aluminum foil and placed in a box with a fluorescent lamp tube (directly exposed to the light) at room temperature.

(c) *Fluorescent light exposure at room temperature (FL-RT):* The samples stored in glass containers were placed in the same box as condition (b).

(d) *Diffused sunlight exposure at room temperature (SL-RT):* The samples in glass containers were placed in same condition as (c).

The effect of temperature on PE degradation was observed from the samples stored under treatments NL-4C and NL-RT. The effect of light exposure and light sources was observed from the samples stored under treatments NL-RT, FL-RT, and SL-RT. The autoclaving eliminated microorganism activity in the pressed seeds compared with no treatment and dried pressed seeds. For the NL-RT and FL-RT

treatments, the fluorescent light exposure was set up to occur in a box. Two fluorescent lamp tubes (Lifemax Super 80, 36 watts, 4000K cool white, 3350 lumens) were attached to the roof or top of the box (0.3 m width, 1.5 m length, and 0.7 m height). The distance between the lamps and the bottom of the box was 0.6 m. The fluorescent lamps were set to be on from 6:00 am to 18:00 pm, for 12 hours of exposure. For the SL-RT treatment, the diffused sunlight in this study was indirect sunlight that shone through the glass window of the experiment room with no control over intensity. The temperature in the room varied between 25°C to 30°C; it was approximately 2°C to 5°C higher for the box with the fluorescent lamps. Light intensity and UV energy flux at the sample position stand in the experiment room is shown in Table 3-5. For diffused sunlight condition, only maximum light and UV intensity during the study were reported.

Table 3-4 Light intensity and UV intensity of Storage condition

Parameters	Storage light room	
	Diffused sunlight[a]	Fluorescent lamp
Light intensity [b] (lx)	1097[b]	4690
with glass cover	997	4340
% light adsorption by Pyrex glassware	9.1	7.5
UV intensity [c] (μW cm^{-2})	20.9	17
with glass cover	18	12.7
% light adsorption by Pyrex glassware	13.9	25.3

Note: [a] the light intensity and UV intensity of diffused sunlight were reported at the maximum value that detected. [b] light intensity in visible light range. [c] UV intensity in 280 – 380 nm range.

3.2.8. First Order Degradation Rate Calculation

In order to quantify PEs degradation rates, the kinetics rates were introduced into this present study. Most degradation rates of chemicals in nature follow first order kinetics [89]. Nonetheless, all degradation results versus time were tested with zero,

first and second order kinetics. The results confirm that the data fit well with first order kinetics (data not shown).

The general first order degradation equation is shown as Equation 3-1. The degradation rate is plotted as the slope of the graph between natural logarithm (*ln)* of PEs in the sample versus storage time (Equation 3-2).

$$C = C_0 exp^{-kt} \qquad \text{Equation 3-1}$$

$$ln[C] = ln[C_0] - kt \qquad \text{Equation 3-2}$$

where [*C*] is the PEs content (mg mL^{-1} from the oil and mg g^{-1} from the pressed seeds) at time *t* , [C_0] is the initial PEs (mg mL^{-1}), *k* is the degradation rate (d^{-1}), and *t* is the storage time (d).

3.3. Analytical Method

3.3.1. Quantitative Analysis of Crude Oil Content by Soxhlet

The oil content analysis was followed AOAC 2006, method No. 920.39a. Twenty grams of sample were extracted by 175 mL hexane in soxhlet apparatus for 12 h then evaporate hexane by evaporator. The oil content was calculated by minus the weight of round-bottom flask after evaporated hexane with the weight of round-bottom flask before extraction then divided with the total sample weight.

3.3.2. Quantitative Analysis of Phorbol esters

Sample preparation

In case of PEs solubilization, the aqueous samples were directly analyzed.

In case of PEs in pressed seeds, the 2 g of the pressed seed were extracted with 20 mL of methanol using a GFL orbital shaker, Model 3017 at 300 rpm for 4 h. This extraction method was verified and found to recover 88.1 % of the PEs [8].

In case of PEs in the oil, 1 mL of oil was extracted with 1 mL of methanol; this step was repeated four times. The extracts were combined and adjusted to a volume of 5 mL.

All sample were filtrated with 0.45 μm PTFE syringe filter before analyze by HPLC (Shimadzu, model LC-100ADvp) using a modified version of the method proposed by Hass and Mittelbach [9]

HPLC condition

The reversed-phase column (octadecyl functional group: Inertsil ODS-3 with 5 μm, 4.6 × 250 mm^2, from GL Sciences, Inc.) was used in the HPLC analysis. The temperature was controlled at 35°C. The mobile phase was an isocratic acetonitrile/water mixture at an 80:20 volume ratio; the flow of the mobile phase was controlled at 1 mL min^{-1}. A UV adsorption detector was used to measure the absorbance at 280 nm. The injected sample volume was 20 μL. A methanol solution of TPA was used as an external standard to establish the calibration curve; the concentrations of the PEs were calculated using this curve. The molecular weights of the PEs were represented as equivalents of TPA required to convert to the mole-solubilization of 616.84 g mol^{-1}.

3.3.3. Quantitative Analysis of Oil Solubilization

The aqueous samples were directly analyzed using an HPLC system equipped with a UV detector. The same reversed-phase column used to analyze the PEs was used. The temperature was controlled at 70°C. An isocratic elution of acetonitrile-isopropanol at a 30:70 volume ratio was applied at a flow rate of 0.75 mL min^{-1}. The UV adsorption detector measured the absorbance at 210 nm. The injected sample volume was 20 μL. A calibration curve was established using *J. curcas* oil dissolved in isopropanol as an external standard. The molecular weight of jatropha oil was 894 g mol^{-1} [90].

3.3.4. Quantitative Analysis of Surfactant

The samples were diluted in ethanol to appropriate concentration and analyzed for the concentration of nonionic surfactant by HPLC using an evaporative light scattering detector (ELSD). A reversed-phase column was used with the

temperature controlled at 50°C. A gradient system was applied using an acetonitrile-water solution. The gradient elution started with 10% acetonitrile for the first 5 min, and the concentration of acetonitrile was increased to 90% at 30 min. The acetonitrile was kept constant for 5 min and was then decreased to 10% for another 10 min. The flow rate was 1 mL min^{-1}. The injected sample volume was 10 µL. The ELSD was set at 40°C and 2.2 bar nitrogen pressure; and the gain signal was 1.

The concentration of anionic surfactant remaining in the solution was analyzed according to the ASTM D3049 - 89(2009) Standard Test Method for Synthetic Anionic Ingredient by Cationic Titration.

3.3.5. Quantitative of Total Nitrogen

The total nitrogen (TN) in the extracted solution was analyzed by TOC analyzer (Shimadzu, model) connected with TN unit (Shimadzu, mode). Injected sample volume was 50 µL. KNO_3 was dried at 105°C for 3hr in oven, kept cool in a desiccator, and prepared with deionized water for the TN standard to establish the calibration curve.

3.3.6. Quantitative Analysis of Crude Protein

The protein in sample was converted to ammonia in the Kjeldahl digestion apparatus and analyzed as ammonia content followed AOAC 2006, method No.955.04D. Then amount of ammonia was converted in the crude protein content.

3.3.7. Quantitative of Electrolyte

The electrolyte was analyzed by IC (Dionex, model) which connected with ED50 electrochemical detector. For cation, the analysis condition was 0.5 mL min^{-1} of flow rate, 11 mmol L^{-1} of H_2SO_4 as mobile phase, 30°C of column temperature control. Column was IonPac CS12 A 4×250 mm. For anion analysis, the IonPac AS19, 4×250 mm, was controlled at 35°C. The mobile phase was KOH at 1.2 mL min^{-1} of flow rate. The gradient of KOH concentration was 1 mmol L^{-1} from start up the sample analysis run to 13 min, increase up to 1.5 mmol L^{-1} at 17 min until 24 min, increase up to 16.2

mmol L^{-1} at 29 min until 34 min, and increase up to 40 mmol L^{-1} at 38 min until 43 min.

3.3.8. Kinematic Viscosity

The surfactant solutions were measured kinematic viscosity by using glass capillary viscometer at interest temperature.

3.3.9. Interfacial Tension

The surfactant solution and Jatropha oil were determined the interfacial tension by the spinning drop tensiometer (Dataphysics Instruments GmbH) at 25°C.

3.3.10. Surface Tension

The surface tensions of each surfactant at different concentrations were measured by the Wilhelmy plate method using a SCAT tensiometer (Dataphysics Instruments GmbH, model DCAT 11) at 25°C.

3.3.11. Statistical Analysis

All experiments were performed in triplicate. The standard deviation for each set was calculated and is shown in the graphic results. The statistical analysis was based on one-way analysis of variance (ANOVA) for the comparison of statistical significance using the SPSS (Statistical Product and Service Solutions) version 17.0 software package to compare different conditions at $p < 0.05$. For the Taguchi and CCRD experimental designs were using the STATISTICA version 10.0 software package.

CHAPTER IV
RESULTS AND DISCUSSION

In order to achieve the main objective of this study, the experimental design consisted of four part: 1) phorbol esters (PEs) solubilization, 2) PEs extraction, 3) PEs recovery, and 4) PEs rate degradation. This chapter presents results of each part respectively. Prior to the first experiment on the solubilization of phorbol esters (PEs), the physical and chemical properties of Jatropha seeds were examined. The seed component is shown in Figure 4-1. PEs target compound in this study were determined for the content in Jatropha pressed seeds, shell and pressed oil as demonstrated in Table 4-1. *Jatropha curcas* crop has wide variations in the morphological characteristics in its stems, leaves, flowers, fruits, and seeds, also physical and chemical compositions [2, 43]. The seeds compositions in this study are in the normal ranges [43]. However, the oil content in seeds is lower than the other research found; 47.25% [59]. Almost 100% of oils contain in the kernels (deshell-seed) [57]. PEs concentration in pressed seeds in this study is not far from in solvent extracted deshell seed (0.8 mg g^{-1}; [53]) and is higher than that of the Non-toxic Mexican variety (0.11 mg g^{-1} in kernel; [54]).

Table 4-1 Physical and chemical properties of Jatropha seeds

Properties	Unit	Value
Average seeds weight	(g per one seed)	0.66 ± 0.09
Kernel mass fraction	(g 100 g^{-1} of seed)	62.83
Shell mass fraction	(g 100 g^{-1} of seed)	37.17
Moisture content in pressed seed	(g 100 g^{-1} of pressed seed)	8.05 ± 0.30
Oil content		
- Seeds	(g 100 g^{-1} of seed)	32.00 ± 0.53
- Pressed seeds	(g 100 g^{-1} of pressed seed)	19.00 ± 0.20
Phorbol esters content		
- Seeds	(mg g^{-1})	2.14 ± 0.12
- Kernel	(mg g^{-1})	3.28 ± 0.19
- Shell	(mg g^{-1})	0.21 ± 0.01
- Pressed seeds	(mg g^{-1})	0.79 ± 0.03
- Oil	(mg mL^{-1})	3.83 ± 0.15

4.1. Solubilization of Phorbol esters

The solubilization behavior of PEs was studied for each individual surfactant solution. In the case of the nonionic surfactant micellar solutions, the effects of the hydrophobic portion (C-chain length) and the hydrophilic portion (EON) of the surfactants and the temperature of the system on the PEs solubilization were evaluated. In the case of the anionic surfactant micellar solutions, the effect of electrolyte addition was evaluated. In case of mixed surfactant, SDS was mixed with each nonionic surfactant. The PEs used in this experiment were obtained from those soluble in jatropha crude oil; thus, the oil in the studied systems behaved as a co-solubilizate. Consequently, the solubilization behavior are described in comparable terms for both PEs and jatropha oil. The property of the PEs and the oil indicates their locus in a micelle which is found corresponding to their solubilization behavior. The MSR, which presents the solubilization capacity of a given organic solubilizate in a given surfactant micellar solution, was measured by varying the concentrations of each individual selected surfactant to confirm the solubilization results. The molar solubilization ratios or MSR of the PEs for each surfactant in this study were then calculated; the results are shown in Table 4-2 (the data used to calculate the MSR are shown in Appendix A, Figure a-2.

4.1.1. Solubilization of Phorbol esters in Nonionic Surfactants

Micellar solutions of the nonionic surfactant series of polysorbate and laureth were prepared as an individual solution at a concentration of 20 mmol L^{-1} to evaluate the effects of the C-chain length on the nonionic surfactant series. The cmc of the both surfactant series are lower than this selected concentration (20 mmol L^{-1}). The HLB of the nonionic surfactant series was also used as a normalized parameter to evaluate its relationship to PEs solubilization. Molar solubilization ratio (MSR) of PEs was calculated for each single surfactant.

4.1.1.1. Effect of the Hydrophobic Portion: Carbon Chain

The C-chain length of the polysorbate series varied from 12 to 18 in this study. The results showed that the greater hydrophobicity resulting from the longer C-chain length (from 12 to 16) tended to significantly increase the solubilization of PEs (p<0.05); however, in the case of polysorbate 80, which has a C-chain length of 18, the PEs solubilization decreased, as shown in Figure 4-1. The one-way ANOVA test of this effect is shown in Appendix b-1. The 18 C-chain contains a single double bond that can be expected to lessen the hydrophobicity of the surfactant. This solubilization result is consistent with and was confirmed by polysorbate 20 exhibiting the lowest molar solubilization ratio (MSR) and polysorbate 40 exhibiting the highest MSR (Table 4-2).

Table 4-2 The properties of the surfactants used in this study

Commercial name	C-chain in hydrophobic part	EON in hydrophilic part	MW[a] (g mol^{-1})	HLB[a]	CMC (mmol L^{-1})	MSR of PEs	
						MSR[b]	R^2
Nonionic surfactants							
Laureth-7	12 (70%), 14 (30%)	7	415.02	12.1	0.07[c] 0.082[d]	0.0060	0.9981
Laureth-9	12 (70%), 14 (30%)	9	503.12	13.4	0.07 [c] 0.1 [d]	0.0087	0.9973
Laureth-12	12 (70%), 14 (30%)	12	591.23	14.6	0.07 [c] 0.14 [d]	0.0095	0.9799
Polysorbate 20	12	20	1228	16.7	0.06 [c] 0.06[a]	0.0092	0.9789
Polysorbate 40	16	20	1277	15.6	0.04 [c] 0.027[a]	0.0111	0.9298
Polysorbate 80	18=1	20	1310	15	0.03 [c] 0.012[a]	0.0105	0.9444
Anionic surfactants							
SDS	12	0	288.38	40	4.2[b] 8.2 [d]	0.0181	0.9897

[a]Value given by the manufacturer. [b]Calculation results. [c]Measured value at 25°C. CMC determination graph is shown in Appendix A. Fig. a-1. [d]Value from [20].

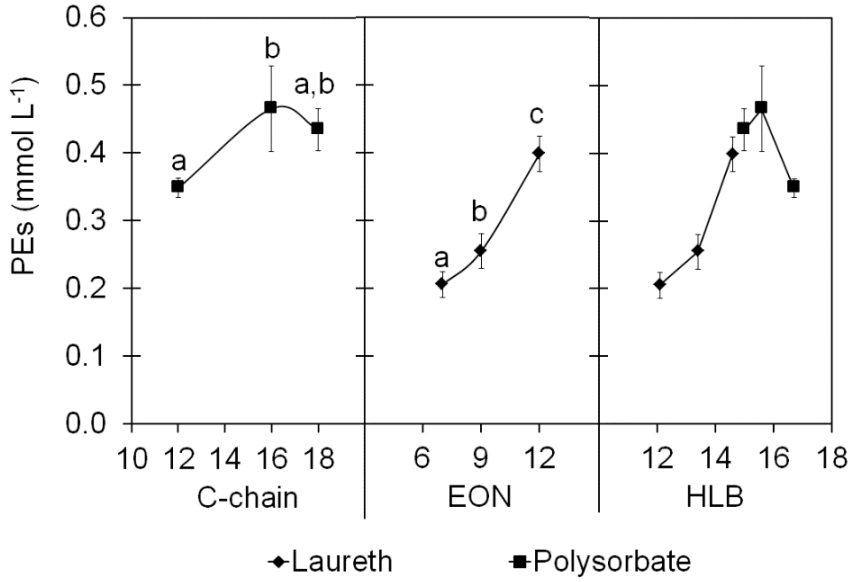

Figure 4-1 Solubilization of phorbol esters in nonionic surfactant solution at 20 mol L^{-1}: effect of EON, carbon chain lengths and normalized HLB. a, b, c is the one-way ANOVA statistical analysis group

4.1.1.2. Effect of the Hydrophilic Portion: EON

The PEs solubilization was significantly increased ($p < 0.05$) with an increase of EON in the surfactants respectively; laureth-12 > laureth-9 > laureth-7 (Figure 4-1). The one-way ANOVA test of this effect is shown in Appendix b-2. This result is in contrast with the result of an increase of the C-length; however, a maximum point was not observed for the various EONs. Nonetheless, the MSR of the laureth series increased with an increasing EON, which corresponds to the solubilization results (Table 4-2). Phasukarratchai et al. [19] observed from the structure of nonionic surfactants that the EON, rather than the carbon chain length of the surfactant structure, significantly enhances PEs solubilization, similar to the extraction of PEs from jatropha pressed seeds. This finding is consistent with the results from the present study that indicated the solubilization of PEs from jatropha oil by the laureth series increased with increasing an EON in the surfactant structure.

Theoretically, both the hydrophilic part, i.e., EON, and the hydrophobic part, i.e., the carbon chain length, can enhance the solubilization of polar organic

compounds [20]. The solubilization of alachlor (a slightly water-soluble molecule) in a nonionic surfactant series with a different EON, Neodol and Triton, exhibited results similar to those of this work in that greater EON values lead to greater solubilization [24]. However, Jafvert *et al.* have reported that, for a nonpolar organic compound, an increased EON of a nonionic surfactant with the same C-chain length decreases the solubilization of hexachlorobenzene [21]. Alam *et al.* reported a similar result, namely, that a reduction in oil solubility occurs with an increased EON of nonionic surfactants [71].

4.1.1.3. Effect of the Hydrophile-Lipophile Balance

In the plot of the HLB of the nonionic surfactants in this study vs. the PEs solubilization (Figure 4-1), the maximum solubilization is observed at approximately 15. The HLB parameter indicates the hydrophilicity and lipophilicity of a surfactant, and it is normalized and comparable for surfactants with different structures. The overall result indicates a linear relationship between the solubilized PEs and the HLB of the surfactant (in the range below 15.6) and then decreased solubilization. Greater hydrophilicity per hydrophobicity of the surfactant and greater HLB parameters tended to result in PEs being dissolved more easily into the aqueous phase and yielded higher concentrations of solubilized polar organic compounds because PEs exhibit a behavior similar to slightly polar organic compounds. This polarity is possibly imparted by the ester and hydroxyl groups in the molecules, as shown in Figure 2-1 in Chapter 2. Figure 4-1 illustrated that HLBs in the range of 15–16 exhibited the highest PEs solubilization. To confirm the conclusion of the PEs behavior in nonionic surfactant solution, the temperature effect and ethanol addition were also studied using laureth-12 and polysorbate 80 solution.

4.1.1.4. Effect of Temperature

Most nonionic surfactants are sensitive to temperature changes [20, 63]. To evaluate the effect of temperature on the solubilization PEs, polysorbate 80 and laureth-12 were selected for study in the range of temperatures below their cloud point (20°C to 60°C). The cloud points of laureth-12 and polysorbate 80 are greater than 100°C and approximately 65°C, respectively. The effect of temperature was clearly observed for the micellar solution of polysorbate 80, but in different directions for the PEs and oil solubilizations. The results showed that higher temperatures cause greater oil solubilization and lower PEs solubilization (Figure 4-2a). However, in the same temperature range (20°C to 60 °C), the micellar solution of laureth-12 was insignificantly ($p < 0.05$) affected with respect to PEs and oil solubilization (see also Figure 4-2a). The one-way ANOVA test of temperature effect in polysorbate 80 system and laureth-12 system are shown in Appendix b-3 and Appendix b-4, respectively. The PEs mole fraction of the total solubilization (oil and PEs) of laureth-12 and polysorbate 80 were calculated by equation 4-1 and exhibit the same trend of the PEs fraction decreasing with increasing temperature (Figure 4-2b). These results correspond to the fact that dehydration of a micelle (palisade) occurred when the temperature increase caused a closely packed palisade region between the EON. Consequently, the solubilization of polar compounds in the micellar solution decreased [20]. Nonionic surfactants are generally more hydrophobic at high temperatures [63]. Increases in the aggregation number of micelles and expansion of the micelle size result in a larger inner core for nonpolar compound solubilization [20]. Thus, the solubilization of the oil is significantly changed, as shown in Figure 4-2a, especially in polysorbte 80 solution. In comparison between the solubilization of PEs and oil, laureth-12 exhibited more PEs selectivity than polysorbate 80.

$$PEs\ mole\ fraction = \frac{PEs\ solubilization}{PEs\ solubilization + Oil\ solubilization} \quad \textbf{Equation 4-1}$$

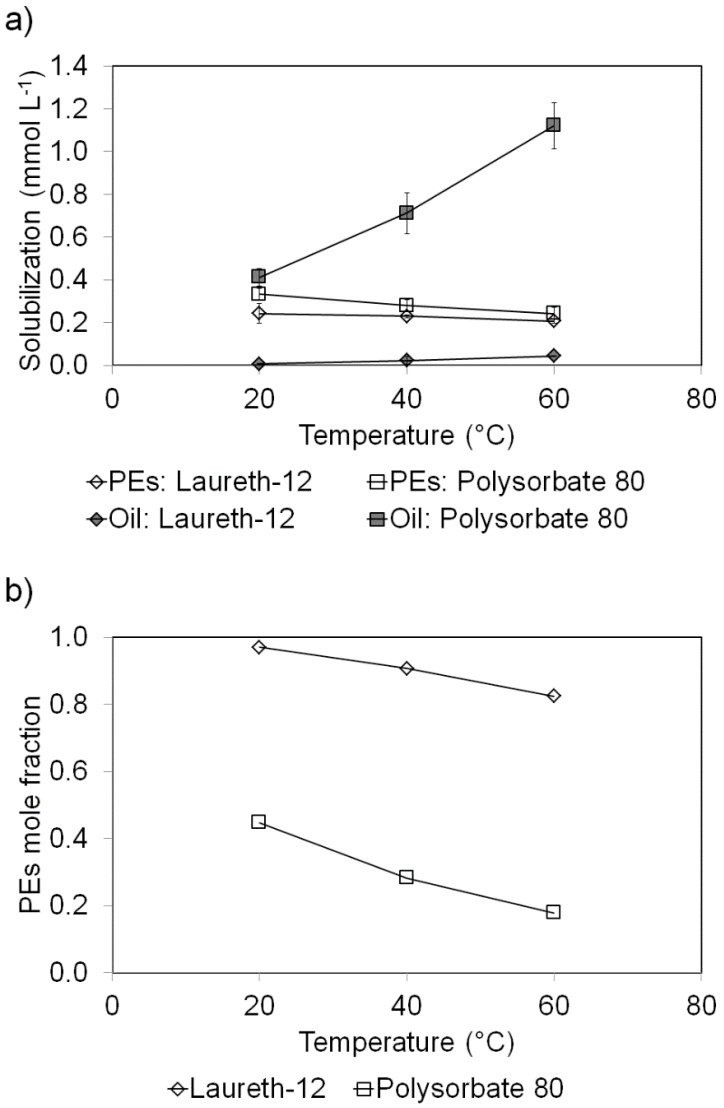

Figure 4-2 Effect of temperature on: a) phorbol esters and jatropha oil solubilization with 20 mmol L^{-1} lolysorbate 80 and laureth-12 solution and b) the phorbol esters' mole fraction from the moles of phorbol esters and oil solubilization.

4.1.1.5. Effect of Ethanol Addition

The addition of alcohol has also been explored as a means to enhance the solubilization capacity of surfactant formulations [20, 91]. Short chain alcohol like ethanol has ability to enhance some polar solubilizates around deeper palisade zone [20]; however, the demicellization is occurred at high alcohol concentration [92, 93]. To evaluate the effect of ethanol addition on the solubilization of PEs and oil, polysorbate 80 and laureth-12 were selected for study in the range of ethanol concentration 0% to 10% volume by volume (% v/v) that below the demicellization point. The results showed that an increasing of ethanol concentration cause singnificantly higher PEs solubilization, while oil solubilizations are not different, for both surfactant solutions ($p < 0.05$) (Figure 4-3a). The one-way ANOVA test of ethanol effect in polysorbate 80 system and laureth-12 system are shown in Appendix b-5 and Appendix b-6, respectively. The PEs mole fraction of the total solubilization (oil and PEs) of polysorbate 80 exhibit the trend of the PEs fraction increasing with increasing ethanol, while no relationship found in case of laureth-12 (Figure 4-3b). Similar to Taylor *et al.* [94], an ethanol addition with 30.5 mmol L^{-1} of polysorbate 80 increase PCE solubilization and decrease solution density. However, ethanol causes the solution viscosity increase. Moreover, Coupland *et al.* [91] found that the molecule flux of solubilization are increased and the IFT is decreased when adding ethanol to polysorbate 20 that cause the solubilization of n-hexadecane increase.

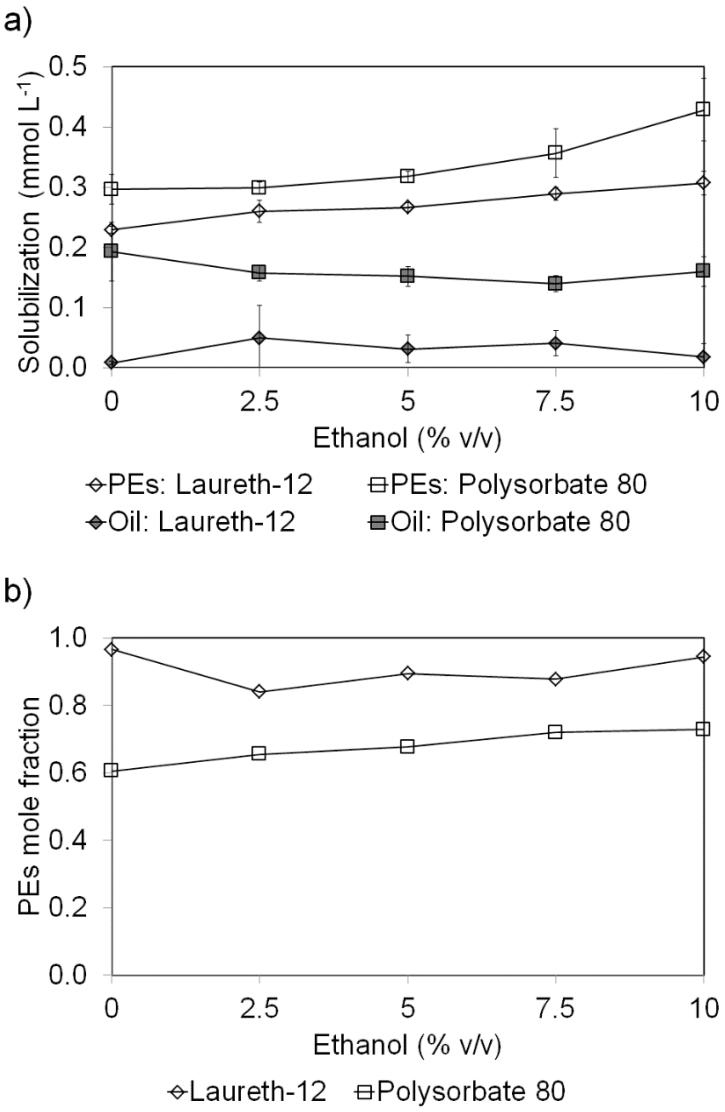

Figure 4-3 Effect of ethanol addition on: a) phorbol esters and jatropha oil solubilization with 20 mmol L^{-1} lolysorbate 80 and laureth-12 solution and b) the phorbol esters' mole fraction from the moles of phorbol esters and oil solubilization.

4.1.2. Solubilization of Phorbol esters in Anionic Surfactant with NaCl Addition

The PEs and oil solubilization in micellar solutions of SDS micelles at different concentrations were evaluated to determine the solubilization capacity of the surfactant in terms of the MSR of PEs (Table 4-1). NaCl was selected for the evaluation an effect of electrolyte on PEs solubilization in anionic surfactant solution. The addition of an electrolyte reduces the electrorepulsive force between the head portion of the anionic surfactants in aqueous solution, thereby reducing the CMC and an increasing the aggregate number of micelles [20, 63]. Within the greater aggregate number of a micelle, the solubilization of an organic solubilizate would be increased. This rule is true in the case of NaCl added to SDS solution, which resulted in an approximately 6-fold lower CMC from 4.2 mmol L^{-1} to 0.7 mmol L^{-1} compared with the CMC of the SDS solution without salt (Figure 4-4). When the concentration of NaCl in the SDS solution increased, the IFT of the system decreased continuously and became mostly steady at 50 mmol L^{-1} NaCl (Figure 4-5a), whereas the PEs solubilization responded inversely to the IFT by increasing until reaching a maximum at approximately 50 mmol L^{-1} NaCl (Figure 4-5b). This result can be explained by the fact that the electrolyte partition between the head group of the surfactant provided a larger palisade area to facilitate the PEs. However, when the electrolyte continued to increase, the palisade layers became closely packed and no longer facilitated the increased PEs solubilization. The jatropha oil solubilization (Figure 4-5b) gradually increased with increasing NaCl without any optimum point until the surfactant solution reached 200 mmol L^{-1} NaCl. This lack of a maximum was due to an increased aggregate number resulting from the NaCl addition, which provided more inner space for the oil. The relationship between the PEs mole fraction (from total PEs and oil solubilization) and the electrolyte concentration (as shown in Figure 4-5b) demonstrated that the expanded micelles resulting from electrolyte addition in the anionic surfactant micelle are more beneficial to jatropha oil solubilization than to PEs solubilization. The results of one-way ANOVA analysis of NaCl effect is shown in Appendix b-7.

As reported by Damrongsiri *et al.* [72], the addition of an electrolyte to an anionic surfactant can enhance the nonpolar compound solubilization in the micelle

core and decrease the solubilization of polar compounds in the palisade layer of the micelles. Ranganathan *et al.* [73] observed that heptane, which is a saturated hydrocarbon, increases the aggregate number of the micelles; i.e., a single micelle contains more surfactant monomer. However, the hydration of the area between the hydrophilic head group of the surfactant is constant. Heptane expands the size of the micelles without inducing a packing effect on the head group of the surfactant; however, when NaCl is added to the SDS solution, the micelle shape changes from spherical to rod-shaped micelles at a 4:1 NaCl:SDS mole ratio [73]. This shape change might be another reason why the PEs' solubilization increased at low NaCl concentrations in SDS solutions but tended to decrease at higher NaCl concentrations: the rod-shaped micelles resulted in a smaller palisade area compared with that afforded by spherical micelles [20] and reduced the space available for solubilization of the PEs.

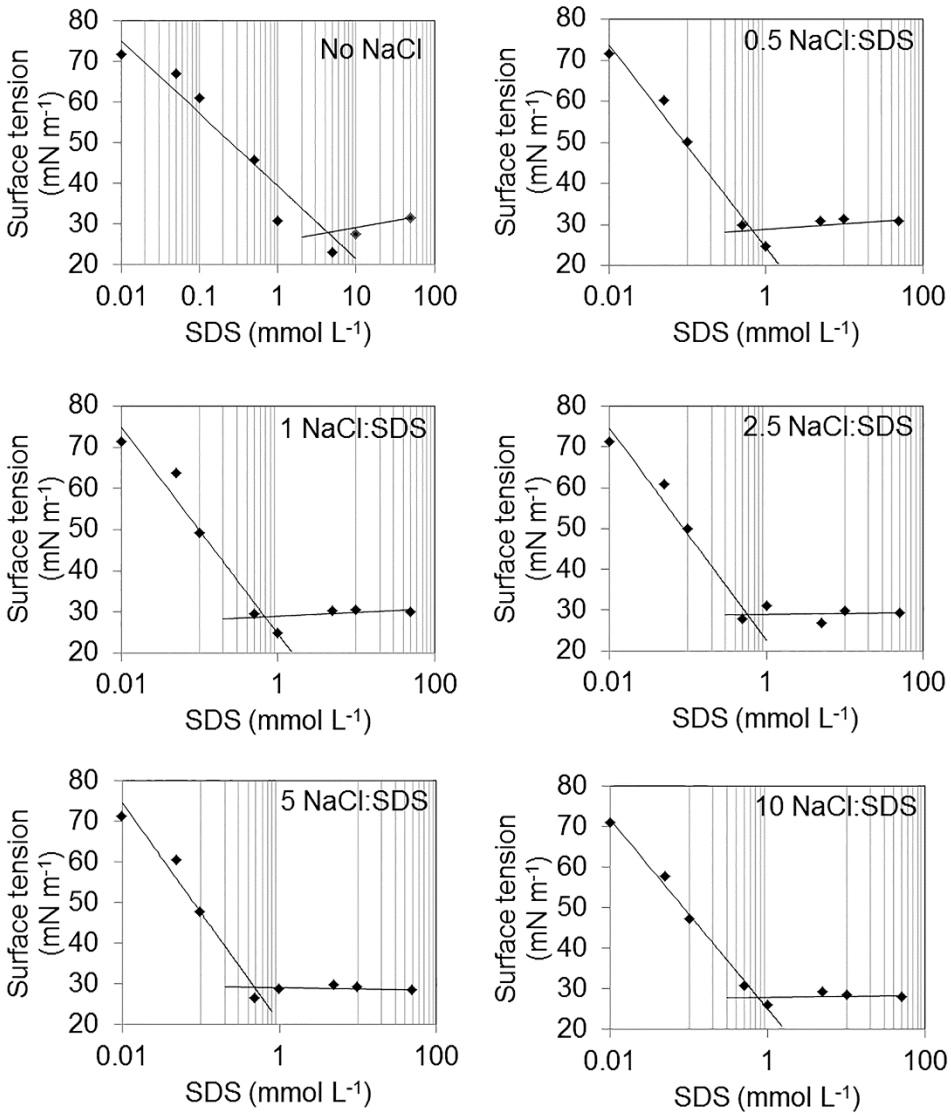

Figure 4-4 CMC determination plot of SDS with effect of NaCl, at 25°C

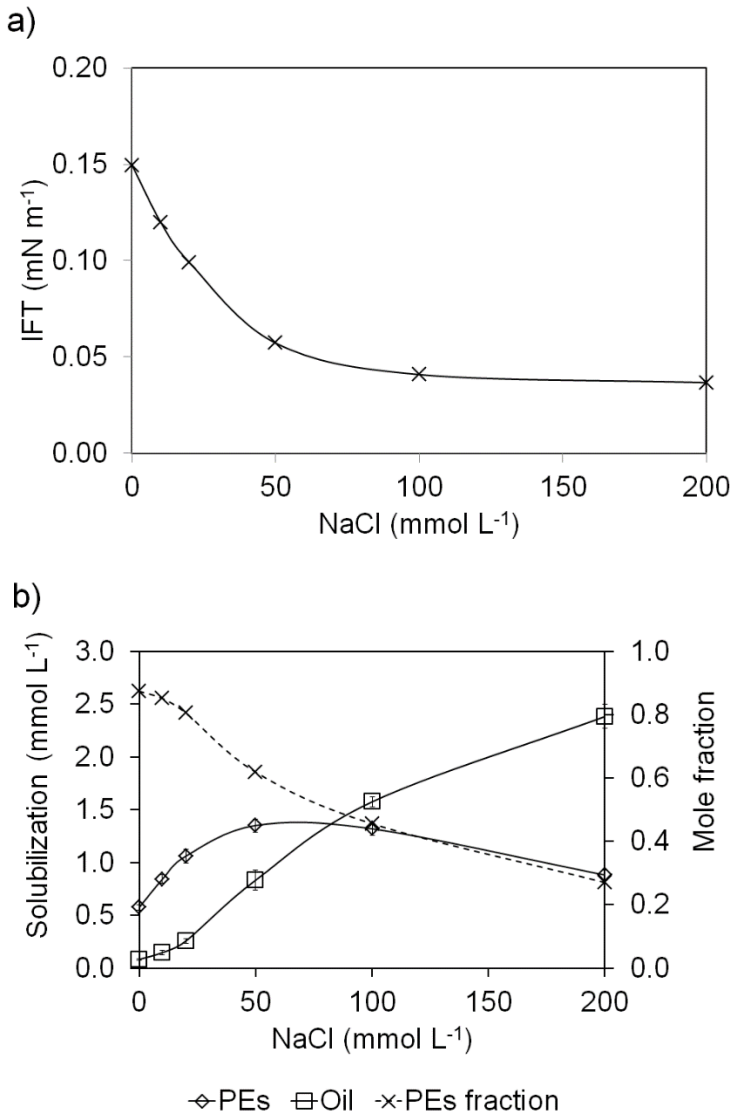

Figure 4-5 Effect of NaCl in 20 mmol L^{-1} of SDS anionic surfactant on: a) the interfacial tension with jatropha oil and b) the solubilization of phorbol esters and oil.

4.1.3. Solubilization of Mixed Surfactants

Mixed HLBs of polysorbate 20/nonionic surfactant (laureth-7, laureth-9, leureth-12, or polysorbate 80) and SDS/nonionic surfactant (laureth-7, laureth-9, leureth-12, polysorbate 20 polysorbate 40 or polysorbate 80) at 20 mmol L^{-1} of surfactant were studied to determine the relationship between the HLB and the PEs' solubilization. In case of polysorbate 20/ nonionic surfactant mixture, only laureth-7 mixing, which the lowest HLB surfactant, can be increased the PEs solubilization corresponded with HLB increase (Figure 4-6). The results were inconclusive for both PEs and oil solubilization of the other surfactant mixture series. The one-way ANOVA analysis of mix HLB on PEs and oil solubilization are shown in Appendix b-8 to Appendix b-11 for mixture of polysorbate 20 with (laureth-7, laureth-9, leureth-12, or polysorbate 80), respectively.

In case of SDS/nonionic surfactant mixture, the increasing SDS fraction causes the mix HLB of solution increasing. The results of these experiments were inconclusive. No relationship between HLB of mixed surfactants system and PEs solubilization was found (Figure 4-7); however, SDS mixing supports the lower HLB nonionic surfactant (laureth-7 and laureth-9) to partition into the aqueous solution instead of evacuate into the oil phase (Figure 4-7). Most SDS remains in the solution. Similarly to Muherei and Junin [25], SDS mixing with TX-100 can reduce TX-100 adsorption on the shale rock and partition into Sarapar 147 (organic oil). The low HLB nonionic surfactant (laureth-7 and laureth-9) can enhance PEs solubilzation when mixed with SDS; unlike higher HLB nonionic surfactants (polysorbate group, and laureth-12) (Figure 4-7). The one-way ANOVA analysis of mix HLB on PEs solubilization are shown in Appendix b-12 to Appendix b-17 for mixture of SDS with (laureth-7, laureth-9, leureth-12, polysorbate 20, polysorbate 40 or polysorbate 80), respectively. In contrast, Shi et al. [27] found that SDS:TX100 has the higher PAHs solubilization than any single SDS or TX100. Moreover, Guo et al. [26] report that mixing SDBS with polysorbate 80 can enhance the less polysorbate 80 adsorption on the soil and increase the pNCB solubility and desorption.

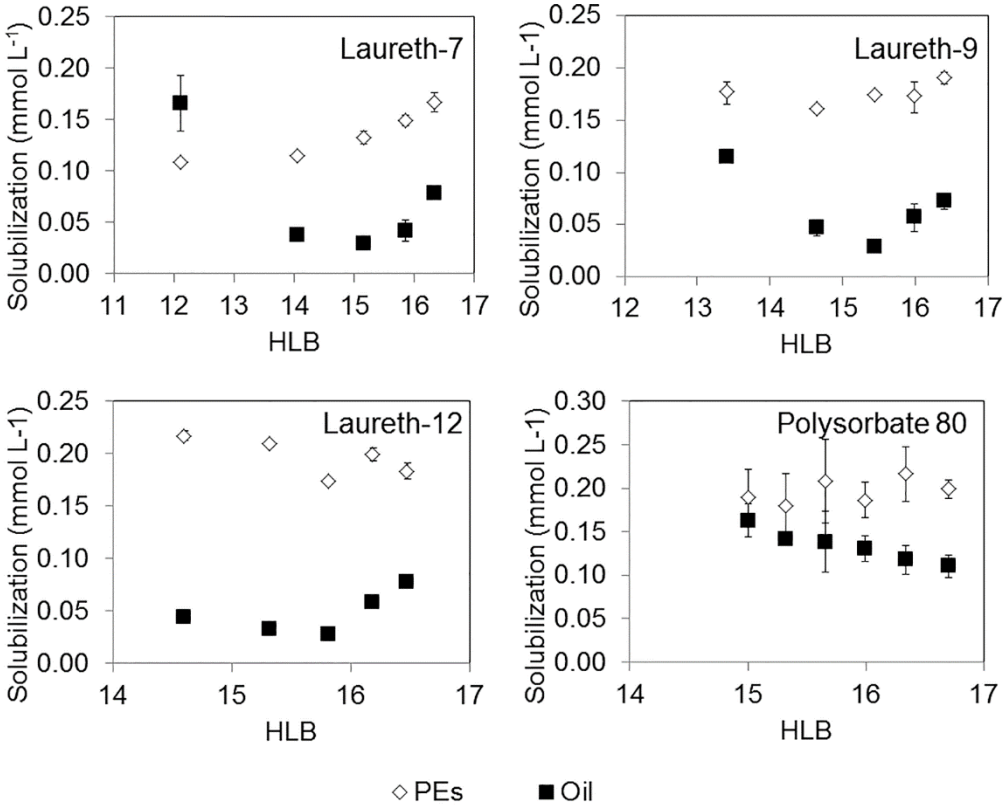

Figure 4-6 Effect of mixed HLB on solubilization of phorbol esters and oil in polysorbate 20 mixed with nonionic surfactant solution at 20 mmol L^{-1} in total concentration

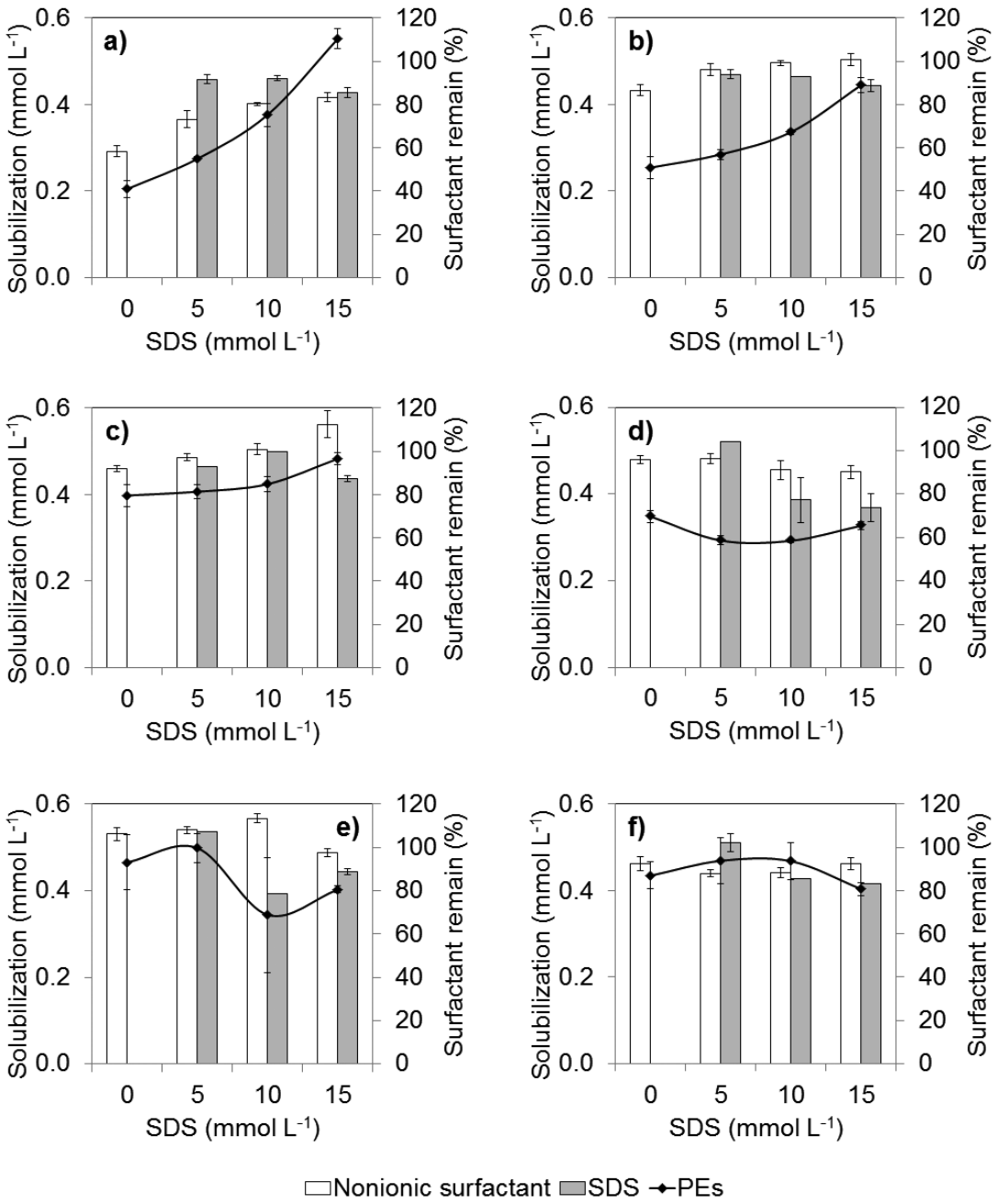

Figure 4-7 Effect of SDS mixed with nonionic surfactants at 20 mmol L^{-1} in total concentration on phorbol esters solubilization and surfactant remaining in aqueous phase solution; a) laureth-7, b) laureth-9, c) laureth-12, d) polysorbate 20, e) polysorbate 40, and f) polysorbate 80

4.1.4. Locus of Phorbol esters in the Micelles

To gain a better understanding of why the solubilization behavior of PEs and jatropha oil in a surfactant micellar solution differs, the locus where the solubilizate exists in the micelle must be identified. The results from the PEs solubilization by nonionic surfactants with different hydrophilic parts (EON) and different hydrophobic parts (C-chain length) and the effect of temperature indicate the locus of PEs in the nonionic surfactant micelle; consequently, the solubilization behavior can be explained on the basis of this phenomenon. When EON, which is the hydrophilic part of the laureth series and usually exists in the palisade region of a micelle, increases, the palisade region is expected to expand and provide more space for polar organic compounds. Consequently, we observed increased solubilization of the PEs when the EON in the surfactant increased. By contrast, when the temperature of the system was increased, the solubilization of the polar compounds(i.e., PEs) decreased [20]. Other researchers have previously investigated the locus of organic compounds that exhibit some functional group bonding in structures similar to PEs. For example, polyaromatic hydrocarbons (PAHs), benzene, and nitrotoluene contain unsaturated bonding in cyclic structures, and phenol contains a hydroxyl group. Kandori *et al.* [74] observed similar results with phenol solubilized within the EO palisade of polyethoxylated nonylphenols. Similarly, Parekh *et al*. [75] reported that phenol is located at the EO of Tetronic 904 (a tetrafunctional block copolymer based on EO and propylene oxide (PO) nonionic surfactant) micelles, whereas benzene is located deeper in PO in the palisade region. In addition, PO is more hydrophobic than EO. Luning-Prak *et al.* [76] demonstrated that nitrotoluene is solubilized into the shell and then into the core of nonionic micelles (polyethylene oxide linear alkyl ether (Brij), polyethylene oxide octylphenyl ether (Triton), and polyethylene oxide alkylphenyl ether (Tergitol) series) when the nitrotoluene concentration is increased. In the case of PAHs, Bernardez [77] demonstrated that PAHs, which are slightly water-soluble, are solubilized into the shell of nonionic surfactants (Brij®35, Brij®30, Tween®80, Triton®X-100 and Tergitol®NP-10) between EO at low PAH concentrations and deep into the core of the micelles at high PAH concentrations. Moreover, Takeuchi *et al.* [23] reported nuclear magnetic

resonance spectroscopy results confirming that naphthalene is located in the palisade region or EO of heptaoxyethoxylated monohexadecyl ether ($C_{16}EO_7$).

In anionic surfactant solutions, electrolyte addition causes a reduction of the solubilized PEs mole fraction. The addition of an electrolyte to anionic surfactant solutions causes packing effects in the head groups of the monomer in surfactant micelles, which reduces the palisade region and reduces polar compound solubilization (27).

Figure 4-8 illustrates the phenomena that are expected to occur when the temperature and electrolyte concentration are increased in the system of nonionic and anionic micellar solutions, respectively. PEs act like large organic compounds with some polar moiety in their structure. The PEs are likely located in the outer palisade region in the EO of the micelles rather than in the inner core in the case of nonionic surfactants and in the outer palisade of the micelles near the anionic head and the first 2–3 carbons of the tail in the case of anionic surfactants. In addition, the oil, which consists of less-polar organic compounds, is likely located in the inner core of the surfactant micelles.

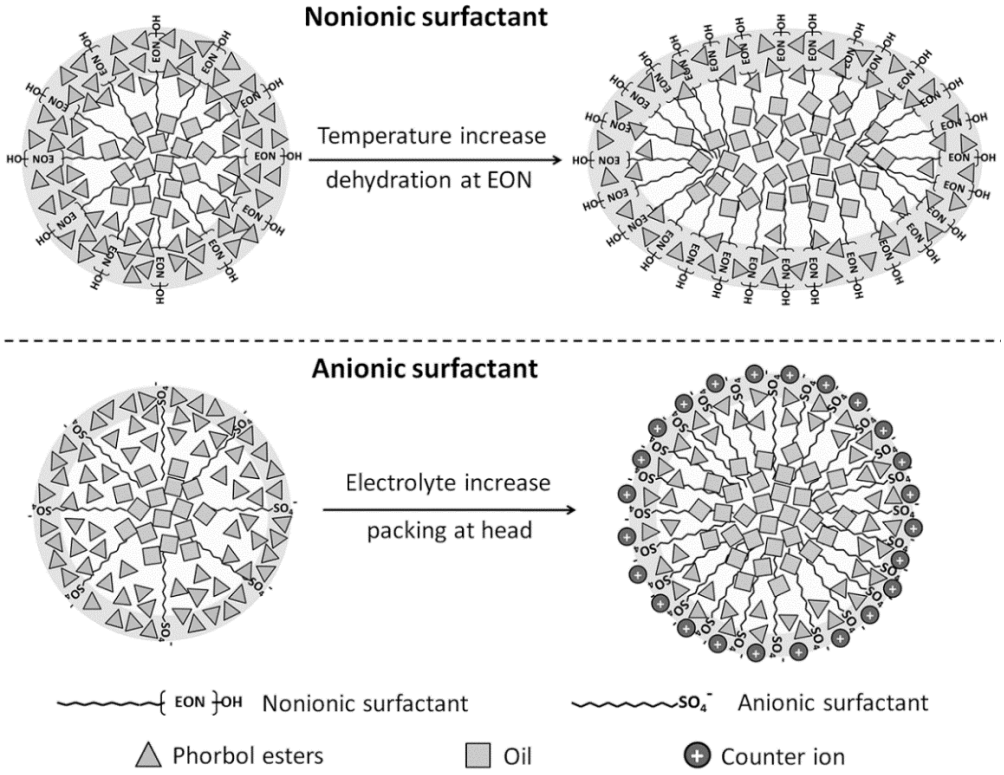

Figure 4-8 Locus of PEs and oil in nonionic and anionic surfactant systems.

4.2. Extraction of Phorbol esters

The PEs extraction from jatropha pressed seeds was investigated to find the suitable system both surfactant solution and physical condition. Firstly, the screening step was studied in order to select some suitable surfactants at fixed physical extraction condition with an orbital shaker. The effect of types and properties of surfactants, and the oil content of the pressed seeds were considered. Then the factors that affect the PEs extraction efficiency were screening by Taguchi statistical analysis. Finally, the optimization of extraction condition was investigated by central composite rotable experimental design (CCRD). The experiments in factors screening step and condition optimization were done in scale-up extraction with an overhead stirrer.

4.2.1. Surfactant Screening

4.2.1.1. Effect of types and properties of surfactants

Generally, the PEs extraction efficiency would be increased by an increase of a surfactant concentration as shown in Figure 4-9a. In the series of nonionic surfactant, EON in the surfactants' structure found to have an effect on PEs extraction efficiency (Fig 4-9a), especially the laureth group. However, for high EON, i.e. laureth-12, polysorbate 20 and polysorbate 80, the effect on the efficiency of PEs extraction found not significantly different (*p=0.05*). The one-way ANOVA of EON effect is shown in Appendix c-1. Moreover, almost nonionic surfactants except laureth-7 were remained in the solution after used more than 80% and 90% for the initial concentration at 20 mmol $L^{-1}$and 40 mmol L^{-1}, respectively (Figure 4-9b). The highest PEs extraction efficiency in Laureth series is laureth-12. In short, for nonionic surfactant solution, the EON in surfactant structure was attributed to PEs extraction efficiency.

In case of anionic surfactant, the PEs extraction efficiencies were significantly (*p=0.05*) lower than the nonionic surfactants: laureth-12, polysorbate 20, and polysorbate 80, especially at lower surfactant concentration (Figure 4-9a). The one-way ANOVA results of surfactant type and of the surfactant solution are shown in Appendix c-2 and c-3. The lower PEs extraction efficiencies are possibly related with the concentration of anionic surfactant remaining in the solution. The anionic

surfactants were losing from the solution more than nonionic surfactants (Figure 4-9b). The surfactant loss resulting by the anionic charge of surfactant and the positive charge of protein in the plant seeds possibly interacted, formed complex, and precipitated from the solution [95, 96]. The PEs extraction efficiencies were increasing with an increasing of the initial anionic surfactant concentration in the solution and the remaining surfactant were increased. Thus, the nonionic surfactants: laureth-12 and polysorbate 80 were selected to find the optimal extraction condition for each surfactant.

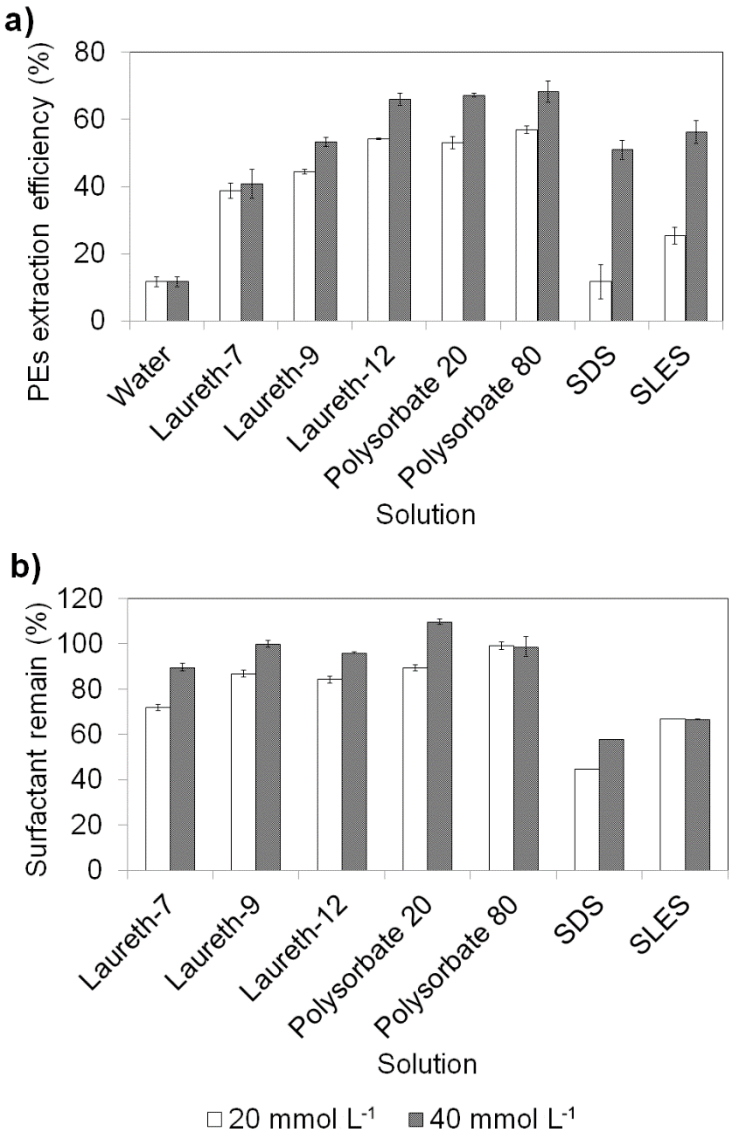

Figure 4-9 PEs extraction using surfactant solution: a) PEs extraction efficiency, and b) surfactant remaining in the extracted solution.

4.2.1.2. Effect of oil content in jatropha pressed seeds

The results in Figure 4-10 show that the oil content in the jatropha pressed seeds had significantly negative effect on PEs extraction efficiency for polysorbate 80 solution ($p<0.05$). In contrast, in laureth-12 solution the oil is possibly become the competitive compounds for PEs extraction. This conclusion can be explained from the main fatty acid compositions in Jatropha oil structure that consists of oleic (C18=1) and linoleic (C18=2) around 70 – 80% with different from the growth location [43, 59]. The unsaturated oil can be more solubilized in unsaturated hydrophobic part of surfactant of polysorbate 80, which is C18=1 chain, than in saturated part of laureth-12, which is C12 chain. The one-way ANOVA analysis of oil content effect is shown in Appendix c-4.

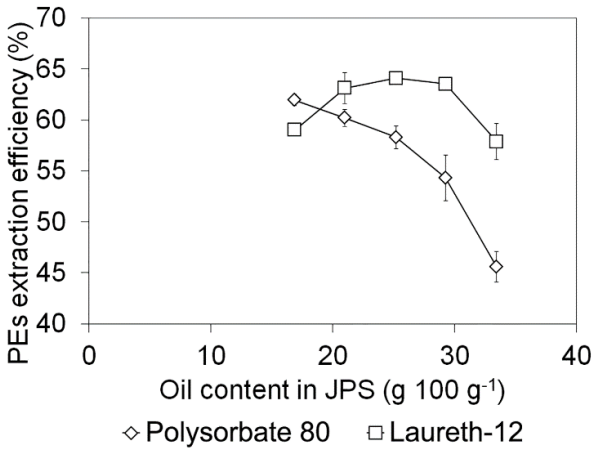

Figure 4-10 Effect of oil content on PEs extraction efficiency by nonionic surfactant solution

4.2.1.3. Effect of surfactant concentration and kinematic viscosity

The kinematic viscosity is one important factor related with the mixing pattern [97] and the mass transfer process [30, 98]. Generally, an increase of noninonic surfactant concentration is result in increase of viscosity of the solution [62]. The kinematic viscosity of the surfactant solution is increased with the increasing of polysorbate 80 concentration in greater degree than that of laureth-12 (Figure 4-11). The viscosity of solution is contributed by the interaction between surfactant and

water. The ethylene oxides (EO) in surfactant enhance the hydrogen bonding between surfactant-water and surfactant-surfactant. A nonionic surfactant which contains higher EON in molecule is showed the higher viscosity properties than the lower EO surfactant [99, 100]. The PEs extraction efficiencies of both polysorbate 80 and laureth-12 solution are sharply increased when the kinematic viscosity of surfactant solution is less than 1 × 10^{-6} m^2 s^{-1} then slightly increase until reach the plateu near 1.3 × 10^{-6} m^2 s^{-1} (Figure 4-11). At the point, the concentration of polysorbate 80 and laureth-12 are 60 mmol L^{-1} (7.86 % w/v) and 100 mmol L^{-1} (7.23 % w/v), respectively, as shown in Figure 4-11. However, laureth-12 shows the higher efficiency than polysorbate 80. The one-way ANOVA of polysorbate 80 concentration and that of laureth-12 concentration are shown in Appendix c-5 and Appendix c-6, respectively.

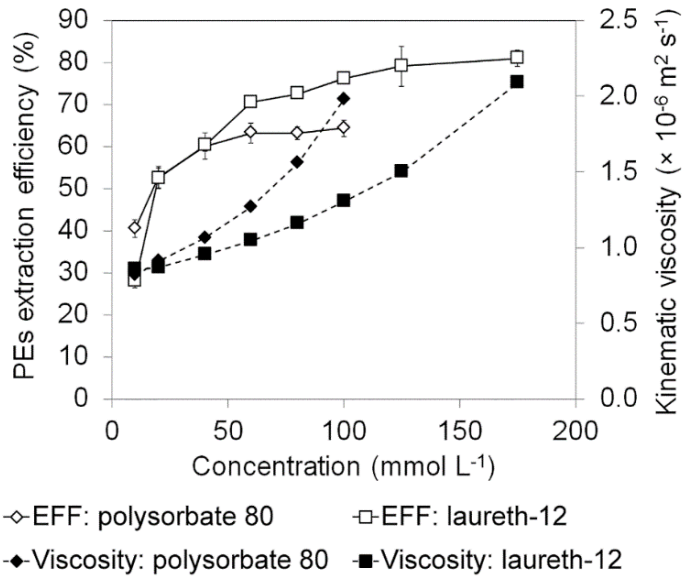

Figure 4-11 Relationship between the concentration and the kinematic viscosity of surfactants and PEs extraction efficiency (EFF)

4.2.2. Screening of Physical Factor for Phorbol esters Extraction

The physical factors in extraction process related with facilitate solvent and solute contact, and mass transfer. Four factors were evaluated with each experimental set of laureth-12 and of polysorbate 80, including of surfactant concentration, extraction time, solid to liquid ratio, and agitation speed. The results show that the all

factors in the study range are significant ($p<0.05$) to PEs extraction efficiency as shown in Table 4-3 and the PEs extraction efficiency of each experimental set is shown in Table 4-4.

In case of laureth-12 solution, the most significant factor when ordering by p value is solid to liquid ratio, followed by surfactant concentration, extraction time, and agitation speed. The highest average value of PEs extraction efficiency of each factor by Taguchi method is 7.5 % of laureth-12 concentration, 45 min of extraction time, solid to liquid ratio at 1 g: 10 mL, and agitation speed at 1,000 rpm that demonstrate as the highest value for each factor in Figure 4-12.

In case of polysorbate 80 solution, the most significant factor is surfactant concentration, followed by solid to liquid ratio, and extraction time; agitation speed is insignificant factor in this study range. The highest average value of PEs extraction efficiency of each factor by Taguchi method is 7.5 % of polysorbate 80 concentration, 30 min of extraction time, solid to liquid ratio at 1 g: 10 mL, and agitation speed at 1,600 rpm that demonstrate as the highest value for each factor in Figure 4-12.

Typically, the speed is higher than 400 rpm is suitable for the diameter of turbine to the diameter of tank ratio (Da/Dt) higher than 0.167 and lower than 1750 rpm for Da/Dt lower than 0.1 [101]. However, the speed of agitation is required (1150 to 1750) rpm for dispersion dry particle in the liquid [87]. The agitation speed extraction from laureth-12 solution and polysorbate 80 solution are followed the guideline. Even though the time and the agitation speed are increase, the scale-up extraction with an overhead stirrer is unable to increase PEs extraction efficiency when extract PEs in jatropha pressed seeds with polysorbate 80. The extraction efficiency of laureth-12 solution is higher than that of polysorbate 80 in all extraction condition. Thus, laureth-12 was selected for extraction condition optimization.

Table 4-3 Statistic results for extraction factor screening by Taguchi method

Laureth-12: Analysis of Variance Mean = 69.3157 Sigma = 7.08683					
Effect	SS	df	MS	F	p
Surfactant concentration (% wt.)	156.3511	2	78.1756	46.9903	0.000017
Time (min)	103.707	2	51.8535	31.1685	0.000090
S:L ratio (g: mL)	532.6049	2	266.3025	160.0709	0.000000
Speed (rpm)	46.1578	2	23.0789	13.8724	0.001781
Residual	14.9729	9	1.6637		
Polysorbate 80: Analysis of Variance Mean = 64.1735 Sigma = 6.90036					
Effect	SS	df	MS	F	p
Surfactant concentration (% wt.)	269.9897	2	134.9949	19.6592	0.000519
Time (min)	136.0448	2	68.0224	9.90606	0.005321
S:L ratio (g: mL)	307.9456	2	153.9728	22.42295	0.000319
Speed (rpm)	33.673	2	16.8365	2.45188	0.141252
Residual	61.8008	9	6.8668		

Note: SS – sum of squares; df – degree of freedom; MS – mean square; F – F test

Table 4-4 PEs extraction efficiency in Taguchi experimental set of laureth-12 and polysorbate 80

Run	Factors				PEs extraction efficiency (%)	
	Surfactant conc. (%)	Time (min)	S:L ratio (g:mL)	Speed (rpm)	Laureth-12	Polysorbate 80
1	2.5	15	1:5	400	53.1 ± 3.7	48.7 ± 1.2
2	2.5	30	1:7.5	1000	68.6 ± 0.2	61.8 ± 4.7
3	2.5	45	1:10	1600	75.3 ± 0.3	67.3 ± 0.6
4	5	15	1:7.5	1600	68.4 ± 0.5	63.9 ± 0.8
5	5	30	1:10	400	73.5 ± 0.2	69.7 ± 3.6
6	5	45	1:5	1000	66.3 ± 0.6	59.9 ± 3.1
7	7.5	15	1:10	1000	77.1 ± 0.3	68.2 ± 3.7
8	7.5	30	1:5	1600	67.0 ± 0.6	67.1 ± 0.6
9	7.5	45	1:7.5	400	74.6 ± 0.0	71.0 ± 0.5

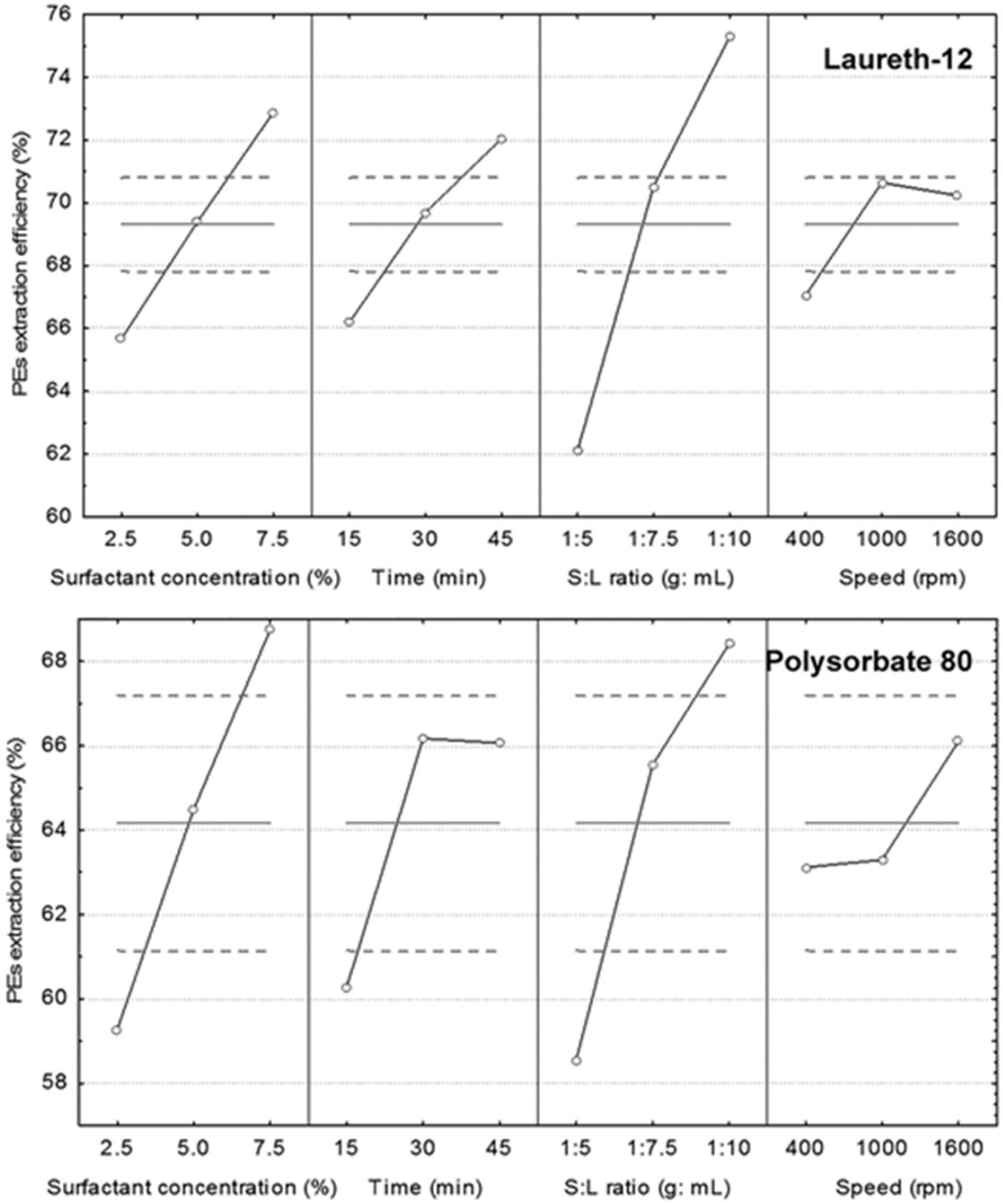

Figure 4-12 Average effect of factors on PEs extraction efficiency from jatropha pressed seeds by Taguchi method (dashed line indicates ±2*standard error)

4.2.3. Extraction Condition Optimization

For the condition optimization, the agitation speed was fixed at 1,000 rpm as shown in the Figure 4-12 that this factor has less positive effect on PEs extraction efficiency when increase speed higher than that point for laureth-12. The increasing of

surfactant concentration, extraction time and solid to liquid ratio potentially increases the PEs extraction efficiency. However, the increase of the solution volume to mass of jatropha pressed seeds will increase the surfactant using amount, thus similar results found in the factor of increasing of surfactant concentration. Therefore, the solid to liquid ratio was decided to fix at 1:10 (g mL^{-1}). Only the concentration of laureth-12 and the extraction time were investigated for the optimization condition. In addition, not only the PEs extraction efficiency was selected as main criteria but also the total nitrogen (TN) in the extracted solution because the TN in extracted solution represents the nitrogen compound of crude protein in the pressed seeds. Thus, the optimal condition for PEs extraction is high PEs extraction efficiency and less crude protein loss from the pressed seeds or low TN in the solution.

The results of PEs extraction efficiency and TN in the solution are shown in Table 4-5. The linear-quadratic main effect model in the CCRD analysis program shows the highest R-square for the results fitting of both PEs extraction efficiency and TN. It demonstrated no interaction between the concentration and the extraction time found for those criteria. Moreover, no lack of fit was found for the model (*p=0.20* for PEs extraction efficiency, and *p=0.13* for TN in extracted solution) that demonstrated the equation generated from the model can use for the prediction in the selected range of the study factors. The statistical analysis on the PEs extraction efficiency and the TN in extracted solution is shown in Table 4-6. Only concentration of surfactant in linear main effect (L) demonstrated the significant positive effect (*p<0.05*) on PEs extraction efficiency and the significant negative effect on TN as shown in Table 4-6. The increase of PEs extraction efficiency depended on the concentration of laureth-12 rather than the extraction time (Figure 4-13); moreover, the increase of extraction time will increase the TN in extracted solution. The critical value for extraction generated by the program is 9.4% of laureth-12 and 51.4 min of extraction time with 1:10 g mL^{-1}, 1000 rpm, and the predicted PEs extraction efficiency is 83.1%. The observed PEs extraction efficiency for single extraction followed the optimal condition is (84.6 ± 0.7) % of PEs extraction efficiency, (87.8 ± 9.0) mg L^{-1} of PEs in solution, while almost (90.8 ± 0.1) % of crude protein remaining in the residual meal. The initial crude protein in the sample was 293.7 g kg^{-1}.

Table 4-5 PEs extraction efficiency and total nitrogen in CCRD experimental set of laureth-12

RUN	Factors		Results	
	Concentration (%)	Time (min)	PEs extraction efficiency (%)	TN in solution (mg L^{-1})
1	2.5	20	72.0	700
2	2.5	60	73.2	711
3	10	20	81.7	509
4	10	60	82.6	550
5	0.9467	40	67.4	760
6	12	40	80.8	493
7	6.25	11.71573	73.5	541
8	6.25	68.3	80.7	631
9 (C)	6.25	40	80.3	608
10 (C)	6.25	40.0	81.1	604

Table 4-6 Effect estimation on PEs extraction efficiency and total nitrogen in extracted solution by CCRD

PEs extraction efficiency (%); R^2=0.94222				
	Effect	Std.Err.	p	Coeff.
Mean/Interc.	80.68	1.20	0.000000	80.68
(1) Concentration (%) (L)	9.50	1.20	0.000525	4.75
Concentration (%) (Q)	-5.68	1.59	0.016059	-2.84
(2) Time (min) (L)	3.07	1.20	0.051469	1.53
Time (min) (Q)	-2.69	1.59	0.151647	-1.35
TN (mg L^{-1}); R^2=0.98251				
	Effect	Std.Err.	p	Coeff.
Mean/Interc.	605.80	11.34	0.000000	605.80
(1) Concentration (%) (L)	-182.40	11.34	0.000017	-91.20
Concentration (%) (Q)	26.51	15.00	0.137526	13.25
(2) Time (min) (L)	44.73	11.34	0.010922	22.36
Time (min) (Q)	-14.29	15.00	0.384577	-7.15

Note: (L) and (Q) is linear effect (x) and quadratic effect (x^2) in the model, respectively. Coeff. is coefficient of factor in equation that generated by Statistica program

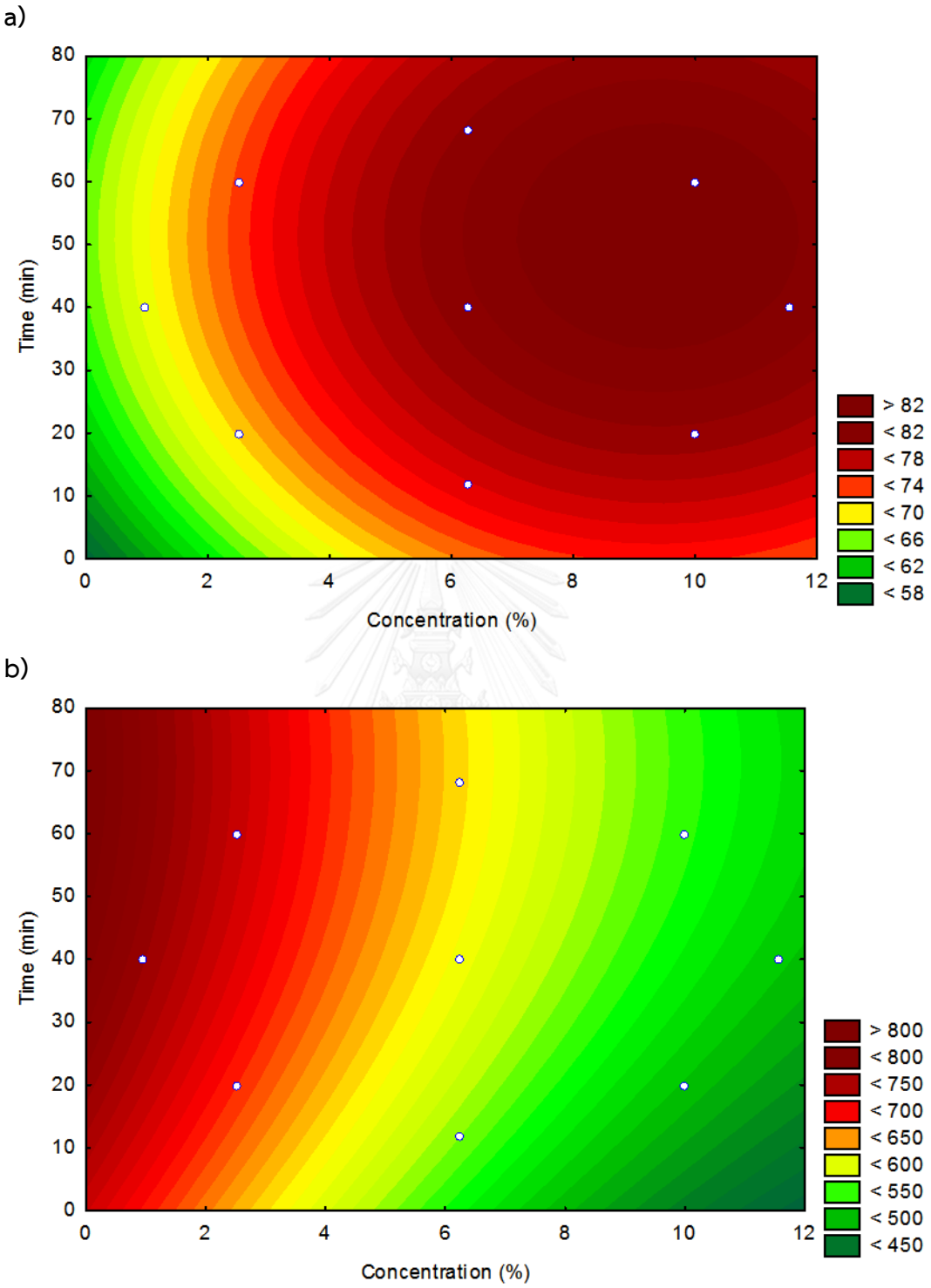

Figure 4-13 Effect of laureth-12 concentration and extraction time on: a) PEs extraction efficiency, and b) Total nitrogen in extracted solution

Extraction time reduction

As in the CCRD statistic results (Table 4-6), the extraction time is insignificant factors that affects the PEs extraction efficiency. The extraction time experiment was studied in order to reduce the time spent with fixed condition as in optimization results, except time. The results show that the PEs extraction efficiency is increasing when increase the extraction time until 40 min, which is not different from 50 min at 95% confident (Figure 4-14). The one-way ANOVA is shown in Appendix c-7. Therefore, the selected extraction condition of 1000 rpm of agitation, 40 min of extraction time, 1:10 g mL^{-1} of solid to liquid ratio, and 9.4% of laureth-12. The PEs extraction efficiency from the selected condition is (82.6 ± 1.6) %, compared with methanol as solvent at same extraction condition shows (81.0 ± 0.8) % of efficiency.

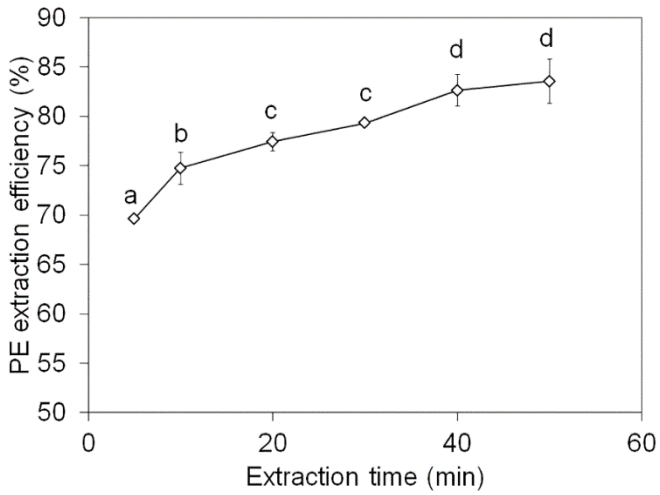

Figure 4-14 Effect of extraction time on PEs extraction efficiency

4.2.4. Reuse Surfactant Solution

In order to concentrate PEs in the extracted solution for PEs recovery process, the single extracted solution was reused as extract solution for fresh jatropha pressed seeds using the same optimal condition for the second and the third extractions. However, the volume of solution was decrease from the start because the pressed seed (JPS) adsorbed water twice time with their total dried weight. The first extraction started with 55 g of JPS and 550 mL of surfactant solution, the solution was remained 450 mL, approximately. Then 45 g of new JPS was extracted with the used solution for

the second extraction, the remaining solution was 350 mL in approximate. In the third extraction 35 g of new JPS was extracted with the second used solution. The solid to liquid ration of overall extraction is 1:4 g/mL. The PEs extraction efficiency in each step and accumulated the PEs concentration in the extracted solution are (72.3 ± 1.4) % and (201.8 ± 19.7) mg L^{-1} for the second time and (55.6 ± 1.3) % and (309.5 ± 19.1) mg L^{-1} for the third time (Figure 4-15). The average PEs extraction efficiency for third extraction is 73%. The third extraction solution was used in the PEs recovery part.

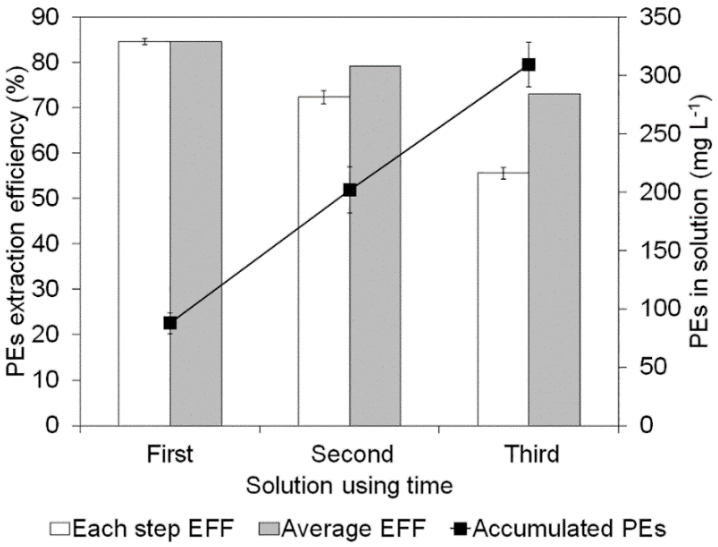

Figure 4-15 Effect of reuse surfactant solution on PEs extraction efficiency and dissolved PEs in solution.

Generally, the jatropha pressed seeds extracted with methanol contain PEs as lower as a non-toxic jatropha variety [12]. However, methanol is considered high toxicity; to reduce methanol by nontoxic or less toxic solvent has been investigated for PEs extraction by several studies. Severa *et al*. [69] used a ionic liquid as co-solvent to reduce the amount of methanol. However, the increasing of co-solvent upper than 60% wt causes the PEs extraction efficiency dramatic decrease due to the increase of viscosity of the solvent. Their optimal extraction condition is 30% wt of ionic liquid per 70% of methanol, solid to liquid ratio of 1:16.5 (w/w), and 22 h of extract time. Almost 98% of PEs was extracted from the jatropha seeds. Guedes *et al*. [15] used ethanol:methanol blending at 50:50 ratio to extract PEs from jatropha pressed seeds. The extraction efficiency reached 97.3%, while the optimal extraction condition was 8

h extract time and solid to liquid ratio of 1:10 (w/v). Even though surfactant solution in this study has lower PEs extraction efficiency (10%) when compared with methanol-based solvent, the extraction time is shorter and no volatile solvent spent in this process.

4.3. Recovery of Phorbol esters

The nonionic surfactant laureth-12 that shows the highest PEs extraction efficiency at the optimal condition was selected to study in this part. Theoretically, solution of POE nonionic surfactant will be separated in two phase known as surfactant-rich phase and dilution phase at above a certain temperature. This phenomenon is called “Cloud point”. As a result of phase separation, solubilizates will also be concentrated in the rich phase [20]. PEs in laureth-12 extraction solution alone has cloud point higher than 100°C that is not practical and high-energy consumption. Therefore, the experiment set up in this part aims to lower cloud point temperature of laureth-12 solution and the PEs extract in this solution with low HLB surfactant additive and electrolytes additive. Moreover, the ability of some solvent to separate PEs from laureth-12 was investigated.

4.3.1. Effect of Laureth-12 Concentration on Cloud Point Temperature

Firstly, the effect of concentration of laureth-12 was studied, NaCl and $CaCl_2$ were added to obtain the variable observed cloud point temperature. The results in Figure 4-16 show that the cloud point was slightly decreased with increase the concentration of laureth-12. Similarly, Talbi *et al*. [102] found the same trend for the ethoxylate (7) fatty alcohol surfactants which has cloud point higher than ambient temperature but lower than water boiling point. The decrease of cloud point of nonionic surfactant which concentration lowers than 10% w/v related with the micelle concentration increase due to micelle-micelle interaction [103]. In contrast, the ethoxylate (3) fatty alcohol surfactant which has cloud point lower than ambient temperature show the increase of cloud point with increase of concentration [102].

Wang *et al*. [36] found that the increase of cloud point due to the concentration of Triton X-45 increase, they concluded that the cloud point of nonionic disperse aqueous solution was different from micelle aqueous solution at above CMC.

When compare between pure laureth-12 solution and PEs-laureth-12 extract solution, the cloud point of pure solution is higher than the extract solution Figure 4-16. The explanation is related with the impurity in the extract solution. Other composition in Jatropha pressed seeds can be extracted into the solution during the extraction process such as ion elements, fatty acid oil, and protein. Those impurity can decrease the cloud point lower than the pure surfactant solution [36, 102-106].

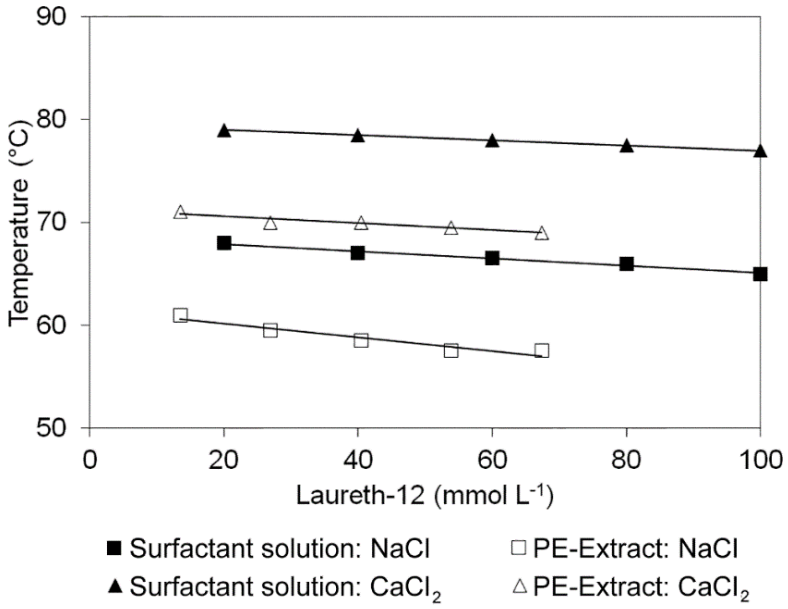

Figure 4-16 Effect of concentration of solution (50 mmol L^{-1} of laureth-12) and PEs-laureth-12 extract solution (33.7 mmol L^{-1} of laureth-12 and 72.6 mg L^{-1} of PEs) on cloud point with fix 2 mol L^{-1} of NaCl and 1 mol L^{-1} of $CaCl_2$

4.3.2. Screening of Low HLB Surfactants Additive

The cloud point temperatures of laureth-12 (50 mmol L^{-1}) solutions are linearly decreased with low HLB surfactant additives fraction and the lower HLB surfactant had higher cloud point lowering ability (Figure 4-17). The lowest HLB surfactant, laureth-1, show the highest cloud point temperature lowing then laureth-2, laureth-3, and laureth-7, respectively. However, with the PEs-extract solution, the similar trend was found at the certain mole fraction of each lower HLB surfactants' addition except in the case of laureth-7 addition (see Figure 4-17). At the additive fraction do not linearly lowered cloud point temperature, the dispersed phase (turbidity) was visually observed on the mixture of extract solution. At the mole fraction of approximately 37% of laureth-1 and laureth-2, and of approximately 47% of laureth-3, the mixing of these additive with the PEs extract are not clear solution at room temperature some are turbid as shown in Figure 4-18. For those samples, when increase the temperature, the turbid turn to translucent, then shift to clear, and after that separation occur.

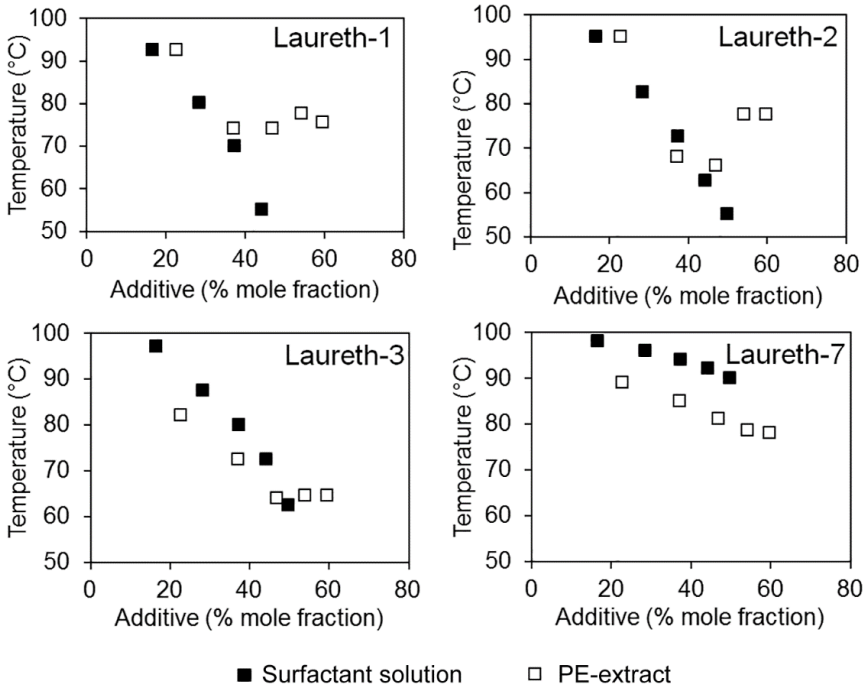

Figure 4-17 Effect of low HLB surfactant additives (laureth-1, laureth-2, laureth-3 and laureth-7) on cloud point of solution (50 mmol L^{-1} laureth-12) and PEs-laureth-12 extract solution (33.7 mmol L^{-1} of laureth-12 and 72.6 mg L^{-1} of PEs)

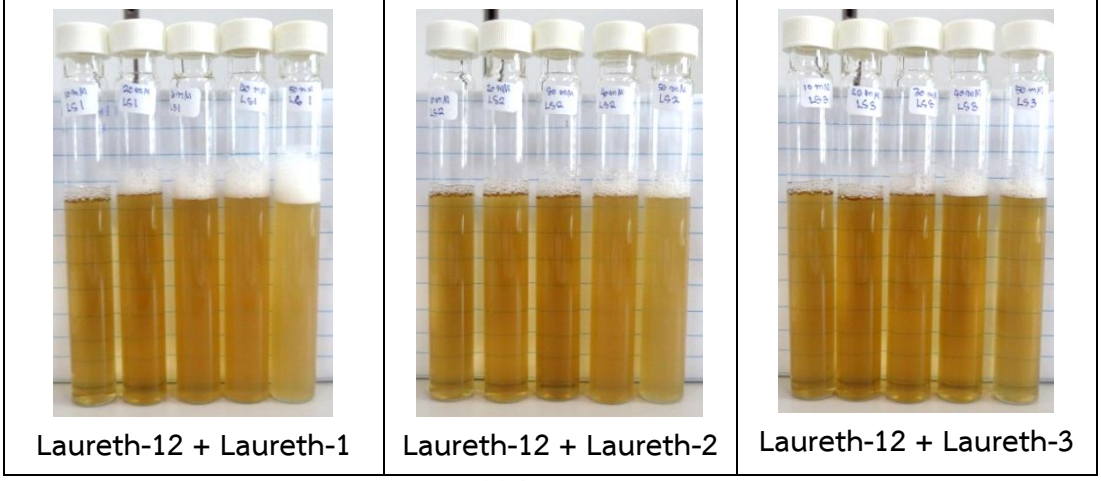

Figure 4-18 Physical observations of low HLB surfactant additives (laureth-1, laureth-2, laureth-3 and laureth-7) mix with PEs-laureth-12 extract solution (33.7 mmol L^{-1} of laureth-12 and 72.6 mg L^{-1} of PEs): the concentration of additives are 10, 20, 30, 40, and 50 mmol L^{-1} from left to right tube in each series.

From this results, the cloud mixture of PE-extract solution with lower HLB surfactants is hypothesized that the cloud point temperature of laureth-12 solution will decrease with low HLB surfactant additive concentration that make the clear solution, but will increase with the additives concentration that make the turbid solution. Similarly, Wang et al. [36] found that the disperse solution of the mixture of Trition X-100 or Triton X-114 with Triton X45 required some temperature to be clear before cloud point phase separation occurred. The increase temperature enhance the solubility of mixed surfactant that call “Krafft point” phenomenon that need more temperature increasing for cloud point [36]. Thus, the laureth-12:laureth-2 mixture and the laureth-12:laureth-7 mixture were selected for further evaluation.

Laureth-12:laureth-2 mixtures (50 mmol L^{-1} of total) are turbid at laureth-2 more than 50%, but all laureth-12:laureth-7 mixtures are clear at every fraction. The results support the hypothesis, the effect of temperature of clear solution followed the cloud point phenomenon but the turbid solution are not shown in Figure 4-19 at laureth-2 above 50% mole fraction. Even though laureth-2 shows high ability to lowering cloud point, the temperature from turbid to separate point is in range of

(5 to 10) °C; however, the rich phase and the dilute phase are not separated well. Thus, NaCl was added to facilitate the density separation between two phases and reduce the temperature gap.

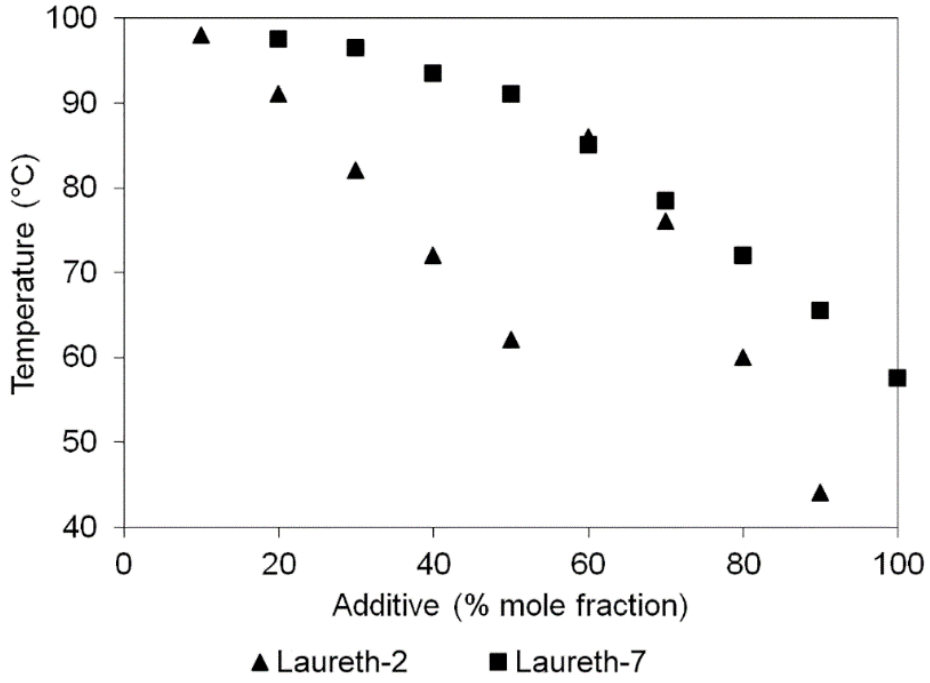

Figure 4-19 Cloud point of laureth-12:laureth-2 mixture and laureth-12:laureth-7 mixture at 50 mmol L^{-1} of total surfactant

4.3.3. Low HLB surfactants additive with NaCl

Some electrolytes are able to dehydrate water from the EO head group of surfactant that decreases the cloud point, called salting out effect [20]. NaCl, known as salting out chemical, was selected to lower the cloud point of laureth-12 solution and PEs-laureth-12 extract solution. Even though NaCl is able to decrease cloud point temperature of the laureth-12 systems (Figure 4-20a), a high concentration of the salt is required which is not really practical. Thus, the addition of both NaCl and laureth-12 were conducted. The results show that the effect of cloud point temperature decreasing occurs due to the combine effect but not synergism both for the pure laureth-12 solution and PEs-laureth-12 extract solution (Figure 4-20a). When focus on PEs in surfactant-rich phase (Figure 4-20b), the percentile of PEs mass recovery from the total mass of PEs in the extract solution is decreased with an increase of NaCl in case of with laureth-2. The system with only NaCl additive is better PEs recovery efficiency than laureth-2 adding system; similar to Talbi *et al.* [102] found that the increase of Na_2SO_4 in cutting oil wastewater can enhance the COD removal efficiency with cloud point separation of nonionic surfactant. Moreover, the concentration of PEs in surfactant rich phase with low HLB additive is lower than without that additive because the volume of surfactant rich phase with laureth-2 is higher than that of without as represented by the height of surfactant-rich phase in Figure 4-20c. Because of the lower PEs recovery and the higher rich phase volume in low HLB additive, only electrolyte additives were interested in order to cloud point temperature lowering and PEs recovery in the next experiment.

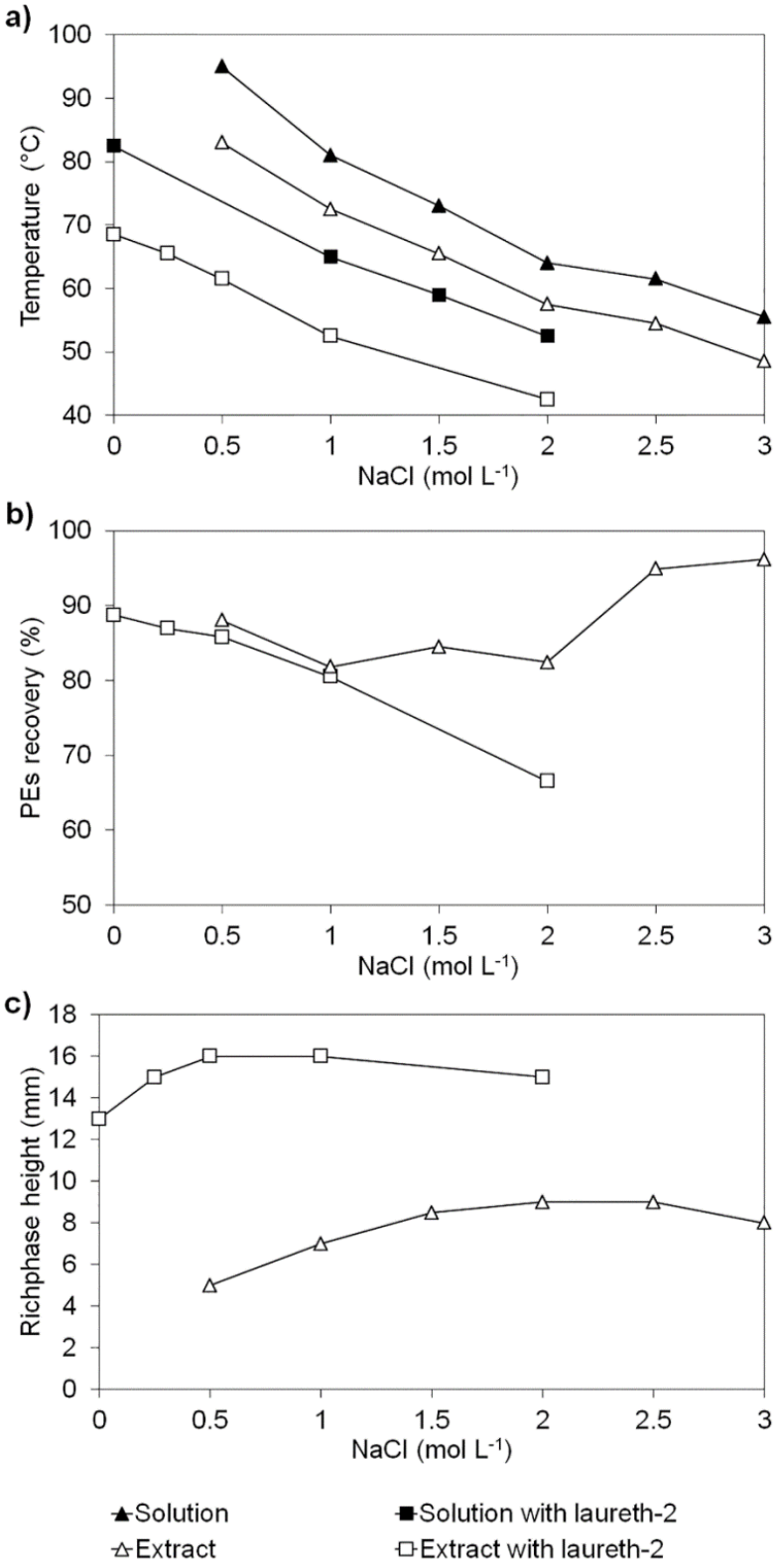

Figure 4-20 Effect of NaCl on: a) the cloud point lowering of solution (50 mmol $L^{-1}$of laureth-12) with and without 20 mmol L^{-1} of laureth-2, b) the mass recoveryof PEs, and c) the height of the surfactant rich phase fraction.

4.3.4. Screening of Electrolyte Additives

The effect of electrolytes on cloud point lowering of 50 mmol L^{-1} of laureth-12 solution was investigated by Chloride-salts series (NaCl, KCl, $CaCl_2$) and, Sodium-salts series of element ion (NaF, NaCl) and of compound ion ($NaNO_3$, Na_2CO_3, Na_2SO_4, Na_3PO_4), respectively. The results show that NaCl, KCl and $CaCl_2$ additive show the same ability to decrease cloud point temperature Figure 4-21. NaF additive show the higher effect on cloud point lowering than NaCl; however, the temperature cannot be lower than 60°C because of the limit of concentration saturation as same as $CaCl_2$. In case of compound ion electrolytes, the cloud point lowering effect is ranking as Na_3PO_4 > Na_2CO_3 and Na_2SO_4 > $NaNO_3$, respectively. The effect of electrolytes was calculated from the linear regression in Figure 4-21 and demonstrates as slope in Table 4-7.

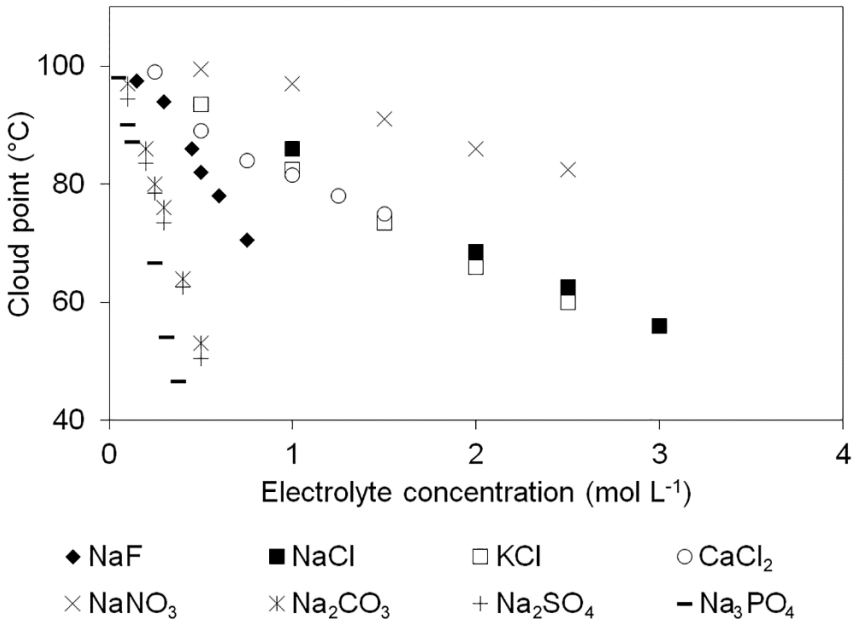

Figure 4-21 Effect of electrolytes on cloud point lowering of 50 mmol L^{-1} of laureth-12 solution

Table 4-7 Linear regression of effect of electrolytes on cloud point lowering

Electrolytes	NaF	NaCl	KCl	$CaCl_2$	$NaNO_3$	Na_2CO_3	Na_2SO_4	Na_3PO_4
Slope	-58.9	-14.2	-16.7	-13.6	-9.7	-109.7	-109.1	-162.2
Y-intercept	113.4	97.7	100.2	95.1	106.1	108	105.6	106.5
R^2	0.9843	0.9689	0.9866	0.9281	0.9864	0.9990	0.9991	0.9977

Note – Cloud point (°C) = Electrolytes (mol L^{-1}) * Slope (°C per mol L^{-1}) + Y-intercept (°C)

The slopes from Table 4-7 refer to the cloud point temperature shift (Δcp) when increases the concentration of electrolyte (ΔCs) as demonstrates in equation.4-2. The salting out effect and cloud point lowering are related with both cation and anion of electrolyte effect [20, 80]. The slope in equation 4-2 is the combination effect of cation and anion in electrolytes. If the shift of electrolytes or ΔCs was fixed as 1 mol L^{-1}, the shift of cloud point was depended on the degree of cloud point lowering of each anion and cation in electrolyte (equation.4-3) and the equation of cloud point shift effect of each electrolytes in this study are expressed in equation.4-3 (a to h).

$$\text{slope} = \frac{\Delta \text{cp}}{\Delta \text{C}_\text{s}} = \Delta \text{cp}_\text{s} \qquad \text{Equation 4-1}$$

$$\text{slope} = \text{No. of cation}\ \Delta \text{cp}_\text{cation} + \text{No. of anion}\Delta \text{cp}_\text{anion} \qquad \text{Equation 4-2}$$

$$\Delta \text{NaF} = \Delta \text{Na}^+ + \Delta \text{F}^- = -58.9 \qquad \text{Equation 4-3(a)}$$

$$\Delta \text{NaCl} = \Delta \text{Na}^+ + \Delta \text{Cl}^- = -14.2 \qquad \text{Equation 4-3(b)}$$

$$\Delta \text{NaNO}_3 = \Delta \text{Na}^+ + \Delta \text{NO}_3{}^- = -9.7 \qquad \text{Equation 4-3(c)}$$

$$\Delta \text{Na}_2\text{CO}_3 = 2\Delta \text{Na}^+ + \Delta \text{CO}_3{}^{2-} = -109.7 \qquad \text{Equation 4-3(d)}$$

$$\Delta \text{Na}_2\text{SO}_4 = 2\Delta \text{Na}^+ + \Delta \text{SO}_4{}^{2-} = -109.1 \qquad \text{Equation 4-3(e)}$$

$$\Delta \text{Na}_3\text{PO}_4 = 3\Delta \text{Na}^+ + \Delta \text{PO}_4{}^{3-} = -162.2 \qquad \text{Equation 4-3(f)}$$

$$\Delta \text{KCl} = \Delta \text{K}^+ + \Delta \text{Cl}^- = -16.7 \qquad \text{Equation 4-3(g)}$$

$$\Delta \text{CaCl}_2 = \Delta \text{Ca}^{2+} + 2\Delta \text{Cl}^- = -13.6 \qquad \text{Equation 4-3(h)}$$

As set the reference value for the effect of cloud point of Na^+ is zero, then $\Delta Na^+ = 0$. The effect of other anion in series of Na^+ was calculated, and the effect of other cations in Cl^- salts was calculated after known calculated ΔCl^-. The calculated values are shown in Table 4-8.

When considered cation type, K^+ shows a small better ability to reduce cloud point because of the negative value of Δcp. In contrast, the Δcp of Ca^{2+} is positive that demonstrate the worse cloud point lowering additive in cation type. In general, divalent of cation increase cloud point [20].

In case of Na^+ salts, F^- show higher negative effect value than Cl^-. Moreover, in case of compound anion element, the highest cloud point lowering salts is trivalent (PO_4^{3-}), following with divalent (CO_3^{2-} and SO_4^{2-}) and monovalent (NO_3^-). The explanation of these results is related with the ionic charge and the ionic size. The

ratio of charge number per ionic radius called ion potential. The ionic potential of anions is ranking from PO_4^{3-} < CO_3^{2-} < SO_4^{2-} < NO_3^-. The higher negative ion potential of the anion shows the higher capability on lowering the cloud point. As comparison with equal valence of Na^+, the highest to the lowest of cloud point lowering effective salts is ranking by the smallest molecular size to the highest one, as similar with other research studies [35, 36, 80, 107].

Table 4-8 Effect cation and anion of electrolytes on cloud point lowering

Ion type	cloud point shift effect, Δcp	Charge number	Ionic radius (Å)	Ion potential
Cation				
Na^+	0	1	1.02 [a]	0.98
K^+	-2.5	1	1.38 [a]	0.72
Ca^{2+}	14.8	2	1.00 [a]	2.00
Anion				
F^-	-58.9	-1	1.33 [a]	-0.75
Cl^-	-14.2	-1	1.81 [a]	-0.55
NO_3^-	-9.7	-1	2.93 [b]	-0.34
CO_3^{2-}	-109.7	-2	2.96 [b]	-0.68
SO_4^{2-}	-109.1	-2	3.09 [b]	-0.65
PO_4^{3-}	-162.2	-3	3.18 [b]	-0.94

Note: [a] Ion radius of elements refer to Shannon [108], [b] Calculated from Ion radius of elements refer to [Shannon [108]], N(3-) = 0.13 Å, C(4+) = 0.16, S(6+) = 0.29 Å, P(5+) = 0.38 Å, and O(2-) = 1.4 Å.

4.3.5. Phorbol esters Concentration by Cloud Point Phase Separation

NaCl, Na_2CO_3, Na_2SO_4, and Na_3PO_4 were selected to study with real PEs-surfactant extract. The approximate concentration of salts was calculated from the cloud point lowering effect equation as in Table 4-7 at fix 60°C that is lower than methanol boiling point (64.7°C). Each salts additive was added with PEs extract solution and observed the phase separation. The results in Table 4-9 show that the volume

fractions of surfactant-rich phase or PEs recovery phase of all electrolytes are not different (p=0.237). The PEs mass recovery is significantly different depending on the electrolyte additive (p=0.002) as same as the laureth-12 mass recovery (p=0.004). The one-way ANOVA results are shown in Appendix d-1.

Table 4-9 PEs recovery results by cloud point separation with selected electrolytes

Parameters	Unit	Additives			
		NaCl	Na_2CO_3	Na_2SO_4	$Na_3PO_4{\cdot}12(H_2O)$
Cloud point	(°C)	50	50	40	50
Rich phase volume	(%)	33.2 ± 1.4^{a}	34.2 ± 2.9^{a}	34.5 ± 0.0^{a}	31.5 ± 1.7^{a}
PEs recovery	(%)	$88.2 \pm 5.2^{c,d}$	80.0 ± 2.2^{c}	91.4 ± 6.1^{d}	70.6 ± 2.9^{b}
Laureth-12 recovery	(%)	93.8 ± 4.3^{g}	$83.1 \pm 4.0^{e,f}$	$87.1 \pm 5.0^{f,g}$	75.2 ± 3.3^{e}
Additive amount	(g/100 mL of extract solution)	15.5	4.6	5.9	10.8
Price of additives	(Bath/g)	0.17	0.74	0.70	1.47
Cost of additives	(Bath/100 mL of extract solution)	2.64	3.40	4.13	15.85

Note: [a] The one-way anova analysis subset of surfactant-rich phase volume results.

[b,c,d] The one-way anova analysis subset of PEs mass recovery results.

[e,f,g] The one-way anova analysis subset of Laureth-12 mass recovery results.

The price of additives is for the analytical grade quality.

The interest parameters including of PEs recovery, laureth-12 recovery, cloud point temperature, surfactant-rich phase volume, and cost of additive were scoring and calculated the normalized score. The score of each parameter is range from 1 to 5. Each range was calculated by the different between the minimum and maximum value. The best condition is the highest PEs recovery, the lowest surfactant recovery, the lowest cloud point temperature, the lowest surfactant-rich phase fraction and the lowest cost of additive. The score range and the weighting factor for normalizing are shown in Table 4-10. The PEs recovery and cloud point were considered at first

hierarchy because they related with the final product yield and the energy consumption in the process. The cost of additive and the laureth-12 recovery were the second because they related with the cost of process. In addition, if the laureth-12 remaining in the surfactant-dilute phase solution is high amount, the surfactant re-adding requirement will less in the reuse solution process for PEs extraction with the jatropha pressed seeds. The total score and the normalized score of each additive are shown in Figure 4-22a and Figure 4-22b, respectively.

Table 4-10 Score range of interested factors and weighting factor

Factor	Score range					Weighting
	1	2	3	4	5	factor
% PEs recovery	70.6-74.16	74.76-78.92	78.92-83.08	83.08-87.24	87.24-91.4	3
%LS12 recovery	93.8-90.08	90.08-86.36	86.36-82.64	82.64-78.92	78.92-75.2	2
Cloud point	50 - 48	48 - 46	46 - 44	44 - 42	42 - 40	3
Rich phase volume	34.5-33. 9	33.9-33.3	33.3-32.7	32.7-32.1	32.1-31.5	1
Cost of additive	15.9 - 13.2	13.2 - 10.6	10.6 - 7.9	7.9 - 5.3	5.3 - 2.6	2

Note: The ranges were calculated from the maximum and the minimum of each factor as show in Table 4-9.

The results demonstrate that Na_2SO_4 is the best both in the highest score and normalized score. NaCl is the second score because the high score in PEs recovery and the low cost or price of NaCl. While, Na_3PO_4 shows only good score in surfactant recovery factor and the total score is equal as the score of Na_2CO_3, this additive normalized score is the lowest.

Na_2SO_4 is the most suitable additive with high PEs recovery and low temperature requirement. The distribution of PEs, laureth-12, Na_2SO_4 and water in surfactant rich phase and surfactant dilute phase were analyzed as shown in Table 4-11. Almost of surfactant and PEs are located in surfactant rich phase and concentrated PEs from 245 mg L^{-1} in initial extract to 649 mg L^{-1} in rich phase. However, the main

composition in this phase is water. Thus, the way to concentrated PEs from rich phase required water removal.

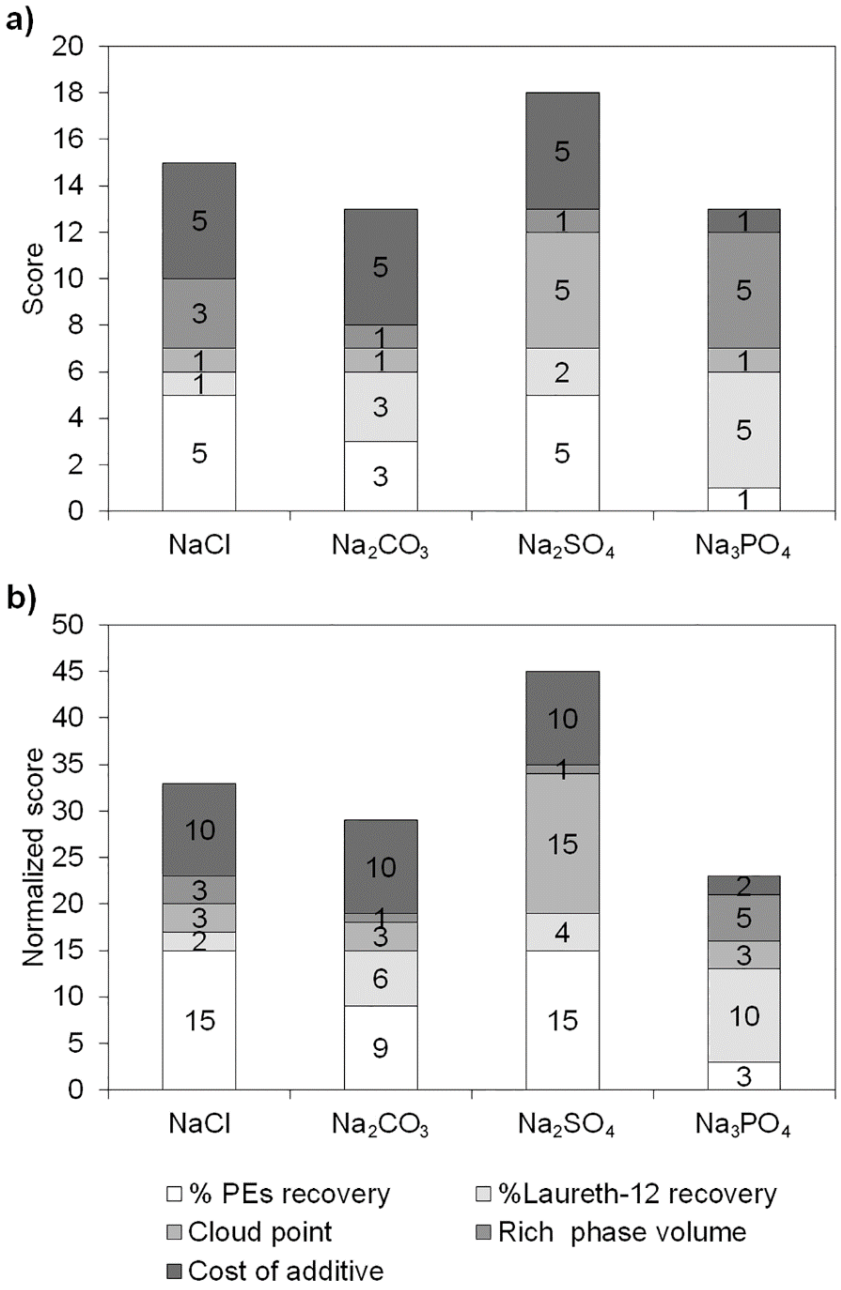

Figure 4-22 Comparison of electrolyte additive of cloud point separation by: a) total score and b) normalized score.

Table 4-11 Composition distribution in cloud point phase separation with Na_2SO_4 additive

Approximate Composition	Rich phase	Dilute phase
H_2O (% w/v)	69.43	91.27
Na_2SO_4 (% w/v)	5.87	8.12
Laureth-12 (% w/v)	24.7	0.61
PEs (mg L^{-1})	649	7

4.3.6. Solvent Extraction

PEs recovery by cloud point technique can only concentrate PEs in surfactant-rich phase, however to recovery PEs as a pure compound, liquid-liquid extraction is required Therefore a liquid-liquid extraction was preliminary conducted. The results show that polar solvents as butanol and dichloromethane are able to extract PEs from water; however, both PEs and the surfactant (laureth-12) were extracted into the solvents (Figure 4-23). These results indicate that only simple technique like liquid-liquid extraction could not work for PEs separation.

To purify PEs for some specific application, advance separation technique i.e, chromatography may be needed. The matrix of PEs concentrated phase derived from the cloud point separation possibly causes the difficulty for further purification process. Nonetheless, high concentrate PEs in aqueous solution containing laureth-12 obtained from cloud point separation is somehow applicable. This is because laureth-12, a linear fatty alcohol ethoxylate (LAE) surfactant is considered as an environmental friendly surfactant [109]. LAE surfactant is quickly bio-mineralized under aerobic condition in saturated subsurface sediment [110], also under anaerobic condition [111]. The average half-life of LAE in the different soil type was 2 day [112]. The toxicity of LAE is decreased with increase of EON [113].

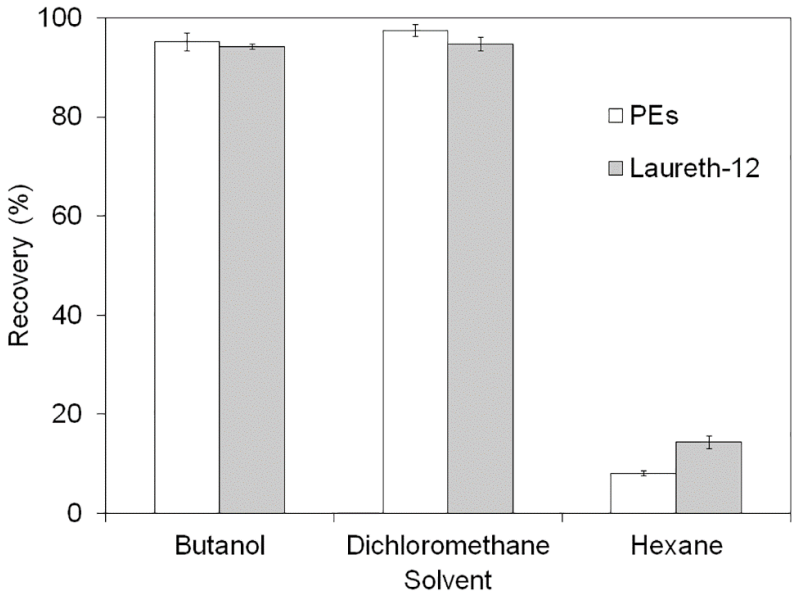

Figure 4-23 PEs and Laureth-12 mass distribution with solvent liquid-liquid extraction

4.3.7. Reuse Surfactant-Dilution Phase Solution

The surfactant-poor phase solution, obtained from PEs recovery process with Na_2SO_4 additive, was tested with the same extraction condition: 1000 rpm, 40 min, and 1:10 solid to liquid ratio. Laureth-12 was added in the solution at 9.4 g per 100 mL. The results show that the reuse solution has the ability to extract PEs lower than the fresh solution around 7.4% and lower crude protein remaining (Figure 4-24). Crude protein in jatropha pressed seeds is (29.37 ± 0.32) %. When considered crude protein loss, the fresh laureth-12 solution added with 5.9% of Na_2SO_4 was studied. Both fresh solution with and without Na_2SO_4 show the same PEs extraction efficiency (p<0.05); moreover, methanol shows no different PEs extraction efficiency from those solution (Figure 4-24). However, the residual meal extracted from the solution with Na_2SO_4 has less crude protein in significant (p<0.05). Moreover, the crude protein remains in meal from both fresh solutions with Na_2SO_4 and reuse solution is not significantly different (p<0.05). Therefore, the impurity of reuse solution causes the PEs extraction efficiency decrease and Na_2SO_4 dissolved in the solution cause higher crude protein loss.

In the PEs recovery process, the extract solutions were separated from the residual meal. Na_2SO_4 need to mix with the extract solution that was fresh solution but no need for the extract solution from reuse and fresh solution with Na_2SO_4. The

temperature requirement in order to concentrate PEs of all surfactant solution is similar; in contrast with methanol, the higher temperature is required for evaporating methanol (Figure 4-24. When considered the PEs concentration, the concentrated factors of each solution were calculated as in the eq.4-4. Methanol show the highest concentrated factor; the cloud point separation shows lower but no significantly different between all surfactant solution condition (p<0.05) (Figure 4-24). The statistic results are shown in Appendix d-2.

$$\text{Concentrated factor} = \frac{\text{PEs concentration in final product}}{\text{PEs concentration in extract solution}} \qquad \text{eq.4-4}$$

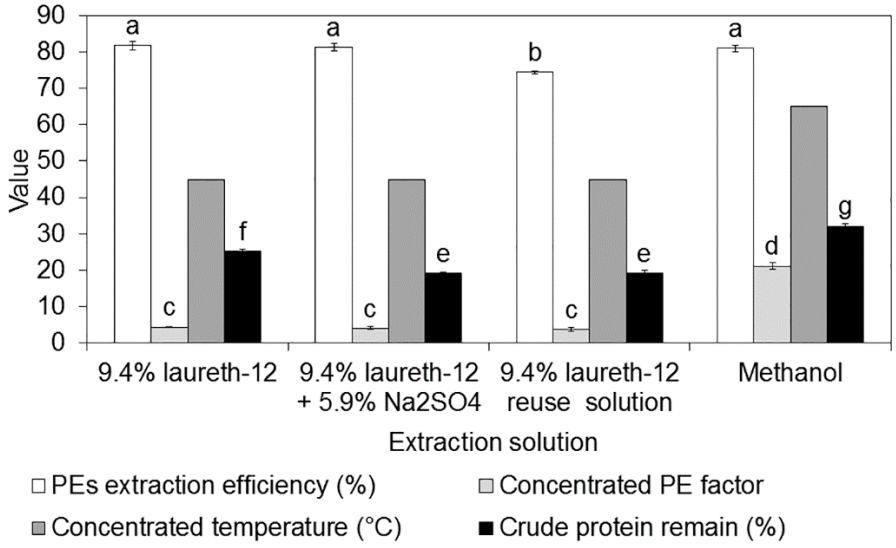

Figure 4-24 PEs and Laureth-12 mass distribution with solvent liquid-liquid extraction

4.4. Natural Degradation of Phorbol esters under the Storage Time

Information on the stability characteristics of PEs in Jatropha oil or pressed seeds provides a crucial basis for further processes PEs extraction processes. Thus, the experiment was designed to mimic practical storage condition to prevent PEs loss. The rate of PEs degradation as affected by light and temperature were then evaluated over a one-year period. The first order kinetic graph for PEs degradation in oil and pressed seeds are shown in Appendix A, Figure a-3 and a-4.

4.4.1. Phorbol esters Degradation in Jatropha Crude Oil

The initial PEs concentration in the oil at day zero was (2.09 ± 0.03) g L^{-1}. PEs in the oil underwent rapid degradation by around (60 ± 3) % within 30 days when exposed to fluorescent light under FL-RT, and (50 ± 4) % when exposed to diffuse sunlight under SL-RT (Figure 4-25). Then, the degradation rate for both treatments declined slightly, and the degradation approached 100% at around day 180 and day 220 for JCO under FL-RT and SL-RT, respectively. JCO kept unexposed to light at 4°C and at room temperature were found to degraded by (24 ± 1) % and (60 ± 2) % by day 360, respectively. The degradation rate *k* values in Table 4-12 2 show that fluorescent and diffused light exposure greatly accelerated PEs degradation in the JCO by factors of 10.5 and 7.4, respectively, compared with the equivalent treatments under the same temperature without light exposure. Oil stored in treatments NL-RT, FL-RT, and SL-RT showed significantly different rates of PEs degradation over the 1-year period ($p<0.05$), indicating the important role of light on the degradation process. Moreover, the light source resulted in slightly different PEs degradation rates, possibly caused by the different light intensities (see Chapter 3, Table 3-5). This result agrees with those in a study by Yunping, Ngoc Ha [38] that shows that close to 100% of PEs in Jatropha oil rapidly degraded within nine days under sunlight (80,000 lx of maximum light intensity) at a room temperature of approximately 25°C. Even though Aregheore, Becker [12] found that PEs are relatively heat-stable over a short period of 30 min at 121°C, the findings of the current study (done over the period of one year) show that without exposure to light, oil kept at room temperature exhibited close to a 280

percent faster degradation rate than oil stored at 4°C (see Figure 4-25 and Table 4-12). The one-way ANOVA results are shown in Appendix E.

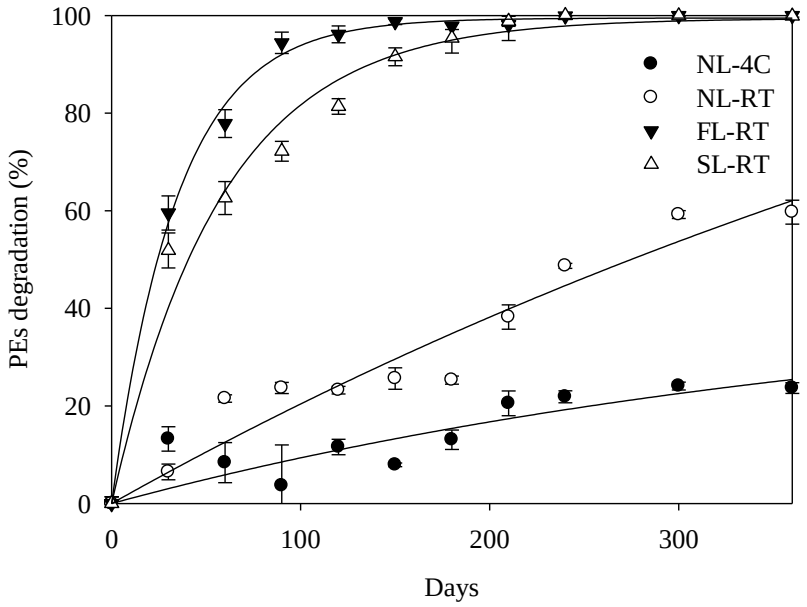

Figure 4-25 Phorbol esters degradation over the oil storage time in the different storage conditions, the initial PEs in the Jatropha oil was 2.09 mg mL^{-1} as TPA equivalent. (NL-4C: non-light exposure at 4°C, NL-RT: non-light exposure at room temperature, FL-RT: fluorescent light exposure at room temperature, and SL-RT: diffused sunlight at room temperature)

Table 4-12 Degradation rates of phorbol esters in Jatropha oil and pressed seeds

Storage condition	Degradation rate, k ($\times 10^{-3}$ d^{-1}) ± 95% CI			
	Oil	Pressed seeds		
	untreated	untreated	dried	autoclaved
NL-4C	0.9 ± 0.01	0.9 ± 0.19	-	-
NL-RT	2.5 ± 0.14	5.5 ± 0.66	4.5 ± 0.29	4.4 ± 0.02
FL-RT	26.2 ± 5.63	6.9 ± 0.57	6.4 ± 0.29	6.0 ± 0.76
SL-RT	18.6 ± 5.96	6.5 ± 0.80	5.0 ± 0.38	5.0± 0.38

4.4.2. Phorbol esters Degradation in Jatropha Pressed Seeds

Three pretreatments of pressed seed samples (untreated, dried and autoclaved) were evaluated for their effects on PEs degradation rates. Since drying and autoclaving effectively eliminated any effect caused by moisture or biodegradation in the seeds, differences between storage conditions could solely be attributed to the effects of light, temperature, and storage time. Four storage treatments were set up for the untreated pressed seeds, while three storage conditions were used for the dried and autoclaved pressed seeds (Figure 4-26). The initial PEs concentrations at day zero in the untreated, dried, and autoclaved pressed seeds were (0.74 ± 0.03) g kg^{-1}, (0.76 ± 0.01) g kg^{-1} and (0.70 ± 0.08) g kg^{-1}, respectively.

While both stored JCO and untreated JPS in treatment NL-4C showed the same *k* value at 0.9 × 10^{-3} d^{-1}, the untreated JPS exposed to fluorescent lighting (FL-RT) and diffused sunlight (SL-RT) were found to have higher *k* values, around 120 percent higher, than the treatments not exposed to the same light source at room temperature. However, no significant PEs degradation difference at $p<0.05$ was found among treatments NL-RT, FL-RT and SL-RT for all three treatment conditions (untreated, dried and autoclaved). This finding indicates that light has much less of an effect on PEs degradation in JPS than in JCO. A possible explanation is that light can penetrate the liquid oil, but not the pressed seeds[114].

However, without light exposure, the PEs degradation rate in untreated pressed seeds was significantly higher ($p<0.05$) compared with that of the oil. The one-way ANOVA results are shown in Appendix E. This may be attributed to the larger surface area of the pressed seed particles; enhanced air diffusion could increase PEs oxidation rates in the seeds. In contrast, there is limited diffusion of atmospheric oxygen into the oil. Thus, PEs present in solid material more readily degraded than PEs dissolved in solvent. This finding is consistent with studies by Schmidt and Hecker [37] and Roach *et al.* [51]. PEs contain many unsaturated bonds, including carbon-carbon double bonds and carbonyl groups (see Chapter 2, Figure 2-1). These weak bonds are readily broken by reaction with oxygen and free radicals [115]. Moreover, molecules containing a larger number of unsaturated bonds require lower energy levels to excite the

electron, so the molecule can be excited at longer wavelengths[116]. Schmidt and Hecker [37] studied the autoxidation of TPA and found that it degraded rapidly at room temperature, especially when exposed to diffused sunlight. Nonetheless, degradation was slow at a lower temperature of 4°C, with no degradation under -20°C. Similarly, Yunping, Ngoc Ha [38] found that rapid PEs degradation in Jatropha oil mixed with autoclaved soil can occur under direct sunlight within six days.

A comparison of untreated pressed seeds, dried pressed seeds and autoclaved pressed seeds indicates that for all treatments, the dried or autoclaved pressed seeds had lower degradation rates than those of untreated seeds (Table 4-12). It was presumed that biodegradation would occur in the untreated pressed seeds and the degradation rate would be much higher in the samples exposed to light. The result corresponds with the same finding in the study of Devappa *et al.* [39] which revealed that PEs degradation rates in soil mixed with Jatropha pressed seeds were temperature- and moisture-dependent, while PEs degradation in the autoclaved samples did not occur under the same incubation conditions.

Moreover, the presence of moisture in the untreated pressed seeds favors biodegradation. Fungal growth was observed in the untreated pressed seeds at four months into the experiment; this was not observed in the dried or autoclaved pressed seeds. This suggests that fungi as well as bacteria may be responsible for biodegradation, consistent with the finding of de Barros *et al.* [42] that PEs from pressed seeds can be degraded to non-toxic levels by white rot fungus under controlled incubation conditions within 30 days. Moreover, da Luz *et al.* [70] found that *Pleurotus astreatus* mushrooms growing on Jatropha pressed seeds can reduce PEs concentrations both in the pressed seeds and mushrooms until PEs concentrations fall to levels below those found in non-toxic Jatropha varieties.

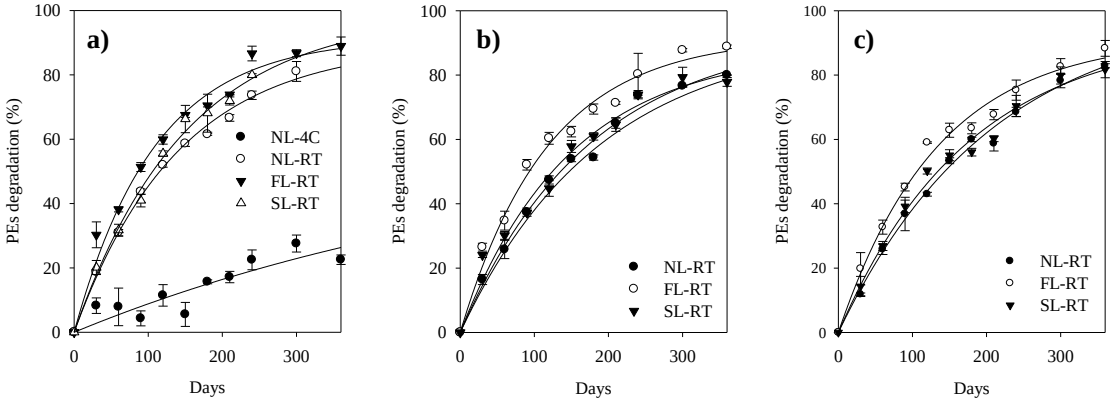

Figure 4-26 Phorbol esters degradation over the Jatropha pressed seed storage time in the different storage conditions, the initial PEs in the untreated (a), dried (b), autoclaved (c) pressed seeds were 0.74, 0.76 and 0.70 mg g^{-1} as TPA equivalent, respectively. (NL-4C: non-light exposure at 4°C, NL-RT: non-light exposure at room temperature, FL-RT: fluorescent light exposure at room temperature, and SL-RT: diffused sunlight at room temperature)

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

5.1.1. Summary of Results

To extract and recovery phorbol esters (PEs) from Jatropha pressed seeds by using surfactant solution, four parts of experimental designed were carried out namely; solubilization of phorbol esters, extraction of phorbol esters, recovery of phorbol esters and natural degradation of phorbol esters. The important results of each parts are concluded as described below:

Solubilization of phorbol esters

- An increase in the EON of the nonionic surfactant molecules has higher effect on enhancing the PEs' solubilization than an increase in the carbon-chain length.
- The hydrophile-lipophile balance (HLB) value was found correlated with PEs solubilization for nonionic surfactant solutions and reveal the optimum solubilization at HLB around 15.
- The PEs mole fraction from the total PEs and the oil solubilization decreased with increasing electrolyte concentration in anionic surfactant solutions.
- The solubilization behavior of the PEs indicates that PEs act more like polar compounds than like nonpolar compounds because the function of ester group, carbonyl group and carbon-carbon double boning in PEs molecule can be contacted with the function of polyoxyethylene in nonionic surfactant molecule.
- The PEs in nonionic micelles are likely located in the palisade region (i.e., between the head group and the first few carbon atoms of the tail), while in anionic micelles are likely near the outer core of the head group.

- Single nonionic surfactant with higher EON has a greater potential to extract PEs from the jatropha pressed seeds.
- An electrolyte at appropriate amount combined with a suitable anionic surfactant as co-surfactant can significantly increases the molar PEs fraction of total solubilization.

Extraction of phorbol esters

- Even though SDS shows similar results to laureth-12 and polysorbate 80 on the PEs solubilization study with jatropha oil. The anionic surfactants are not suitable for PEs extraction from jatropha pressed seeds because of low extraction efficiency from JPS and high surfactant loss from the solution.
- For laureth-12 solution, the optimal extraction condition of PEs from jatropha pressed seeds is 1000 rpm of agitation, 40 min of extraction time, 1: 10 g mL^{-1} of solid to liquid ratio, and 9.4% of laureth-12 solution. The PEs extraction efficiency from the selected condition is 82.6% ± 1.6% that comparable with methanol solvent extraction.
- The extracted solutions can reusable in the process with fresh pressed seeds to increase the amount of PEs in the surfactant solution before recovery process. However, the limitation of efficiency drop should be considered.

Recovery of phorbol esters

- The PEs-nonionic surfactant extract solution can be concentrated by cloud point separation with some additives at temperature lower than 60 °C.
- The low HLB surfactants, found capable to be an additive for lowering cloud point temperature; however, the volume ratio of surfactant rich phase to total PEs-extract was higher than the electrolyte additive alone.
- The effect of factors lowering cloud point temperature is depended on the concentration and ionic strength of electrolytes.
- Trivalent of Na_3PO_4 has higher ability than divalent of Na_2SO_4 at the same molarity basis.

- Na_2SO_4 provide the highest efficiency at 91.4% of the PEs recovery, rich-phase volume, cloud point temperature, and salts amount.
- The final product consists of water as the main component, laureth-12, Na_2SO_4 and phorbol esters as illustrated in Figure 5-1.

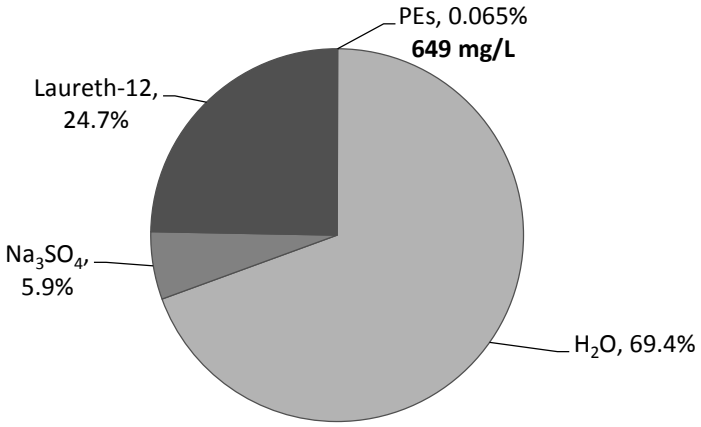

Figure 5-1 Component in final product from phorbol esters recovery process (in the rich phase)

- The dilute phase solution after PEs recovery process can be reused in extraction process again but laureth-12 is needed to add up. The PEs extraction efficiency of the reused solution is up to 74%. No salting out additive is required in the cloud point separation process for reused solution because Na_2SO_4 is remaining in the dilution phase.

Natural degradation of phorbol esters

- Temperature and light exposure are the main factors responsible for PEs degradation in jatropha pressed seeds and jatropha crude oil over one year.
- PEs degradation in both oil and pressed seeds stored at 4°C was reduced compared with room temperature.
- Exposure to fluorescent or diffused sunlight was found to greatly accelerate PEs degradation in jatropha oil.
- When stored at 4°C and protected against any light exposure, PEs degradation of about 24% after 1 year was found in both pressed seed and oil.

5.1.2. Overall Conclusion

The target of this work is to challenge a common approach of PEs extraction using methanol by introducing an alternative approach using surfactant solution. In order to achieve the target, three steps of experimental designs namely; PEs solubilization, PEs extraction, and PEs recovery were carried out and the results are summarized as described earlier. From the experimental results of the overall study, the single laureth-12 solution is recommended for PEs extraction process due to the selectivity, the extraction efficiency and the remaining in solution; moreover, it can be separated by heating with sodium sulfate additive. Solid residual was then separated from the extract solution before cloud point recovery. The result of the PEs extraction and the PEs recovery by the surfactant solution and methanol extraction techniques are summarized and compared as shown in Table 5-1.

In addition, to complete the process for further application, PEs degradation in different storage condition were also studies. This is the first time that PEs degradation in the raw materials form (jatropha crude oil and pressed seeds) was investigated for their degradation. It is recommended that jatropha pressed seeds should be stored at a cool temperature below 4 °C without light exposure if PEs recovery is intended. PEs should be extracted from the raw materials as soon as possible to maximize recovery rates and reduce storage space requirements.

Table 5-1 Comparison between laureth-12 solution and methanol for PEs extraction from Jatropha pressed seeds

Factor	9.4% Laureth-12	Methanol
Extraction		
• Solvent	9.4% Laureht-12 in water	Methanol
• PEs extraction efficiency * (%)	82 ± 1	81 ± 1
• Crude protein in residual JPS (g kg^{-1})	252 ± 5	321 ± 5
• Extraction time (min)	40 for single extraction	40
Recovery		
• Method	Cloud point	Evaporation
• Additive	5.9% Na_2SO_4	No
• Temperature requirement (°C)	40 – 45	> 65
• Recovery time (min)	10	45
• PEs concentration factor	4.34 ± 0.2	21.1 ± 0.9
• Hazard	Non hazard	Flammable Toxic Health risk
Cost of materials (Baht per L of solution)		
• Solution for extraction	18.22	150
• Additive for recovery	41.3	

Note: The extraction with both laureth-12 solution and methanol are done under same physical condition: 100 g of pressed seed per 1 L of solvent, 1000 rpm of agitation speed, 40 min of extraction time. The cost of materials is calculated based on price of materials used in lab experiment scale.

5.2. Recommendations for Further Application and Study

- Even though cloud point separation is able to enrich or concentrate PEs in surfactant rich phase, purified PEs from surfactant matrix possibly will be required for further application. If high purity of PEs is essential such as in medicine application, the other advanced separation technique such as chromatography is required.
- In case that PEs is applied as pesticide, an emulsifier has to be mixed with pure PEs before application because it is immiscible in water. However, the surfactant in our final product shows the same role of emulsifier to facilitate PEs dissolving in water, so it possibly directly applies for pest control application. Moreover, this surfactant is eco-friendly and mostly biodegradable under aerobic condition.
- The phorbol esters residual in the treated pressed seeds should be removed and tested for the toxicity if the further application is related feed stock production.
- To reduce the step of oil extraction and detoxification process (from mechanical press), the suitable surfactant solution and details of extraction method are major concerns in order to combine the extraction of JCO and PEs or protein from jatropha seeds in one step process. Nonionic surfactant solutions that are able to form Winsor type III microemulsion with the JCO should be further study in more details on PEs and JCO extraction to from solid phase of jatropha seeds. These would be expected that the JCO will be separated as excess oil phase and PEs will be solubilized in surfactant aqueous solution layer.
- The criteria of surfactant selection for extraction are the properties of target compound (structure and polarity), the properties and toxicity of surfactants, the matrix component in sample, the purpose of further application, and etc. For example, nonionic surfactants or anionic surfactant extended with POE are more suitable in the system that has high ionic strength and charge surface

area. If structure of target compound is known, the surfactant which contains some similar structures should be selected.

- If structure of the interest target compound is unclear, the solubility study in some designed solvent is recommended. A compound which exhibits higher solubility in non-polar solvent than polar solvent tends to be solubilized in the inner core of surfactant and the solubilization can be enhanced using the longer carbon chain length of surfactant molecule. However, some compound is soluble both in polar and non-polar solvent, for example, PEs is soluble in methanol, ethanol, butanol, dichloromethane and hexane. The effect of hydrophobic and hyphophilic part of surfactant on solubilization is an effective method for screening the suitable surfactants. Four surfactants in same series are required at least. A pair of same hydrophobic structure which different ethylene oxide number (EON) demonstrate the effect of hydrophilic properties. A pair of same EON which different carbon-chain length demonstrate the effect of hydrophobic properties.

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APPENDIX

Appendix A Supplementary results

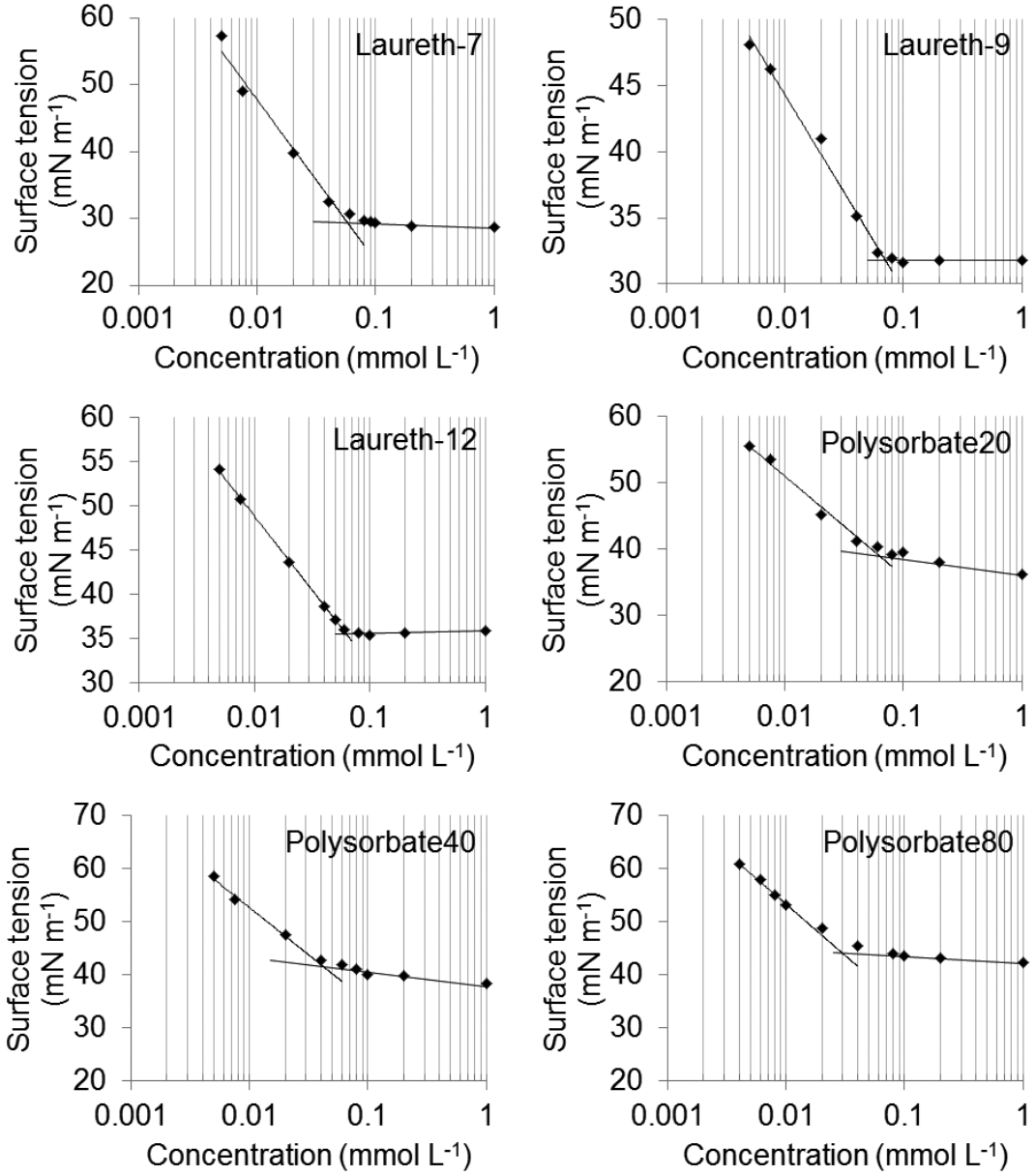

Figure a-1 CMC determination plot of nonionic surfactants, at 25°C

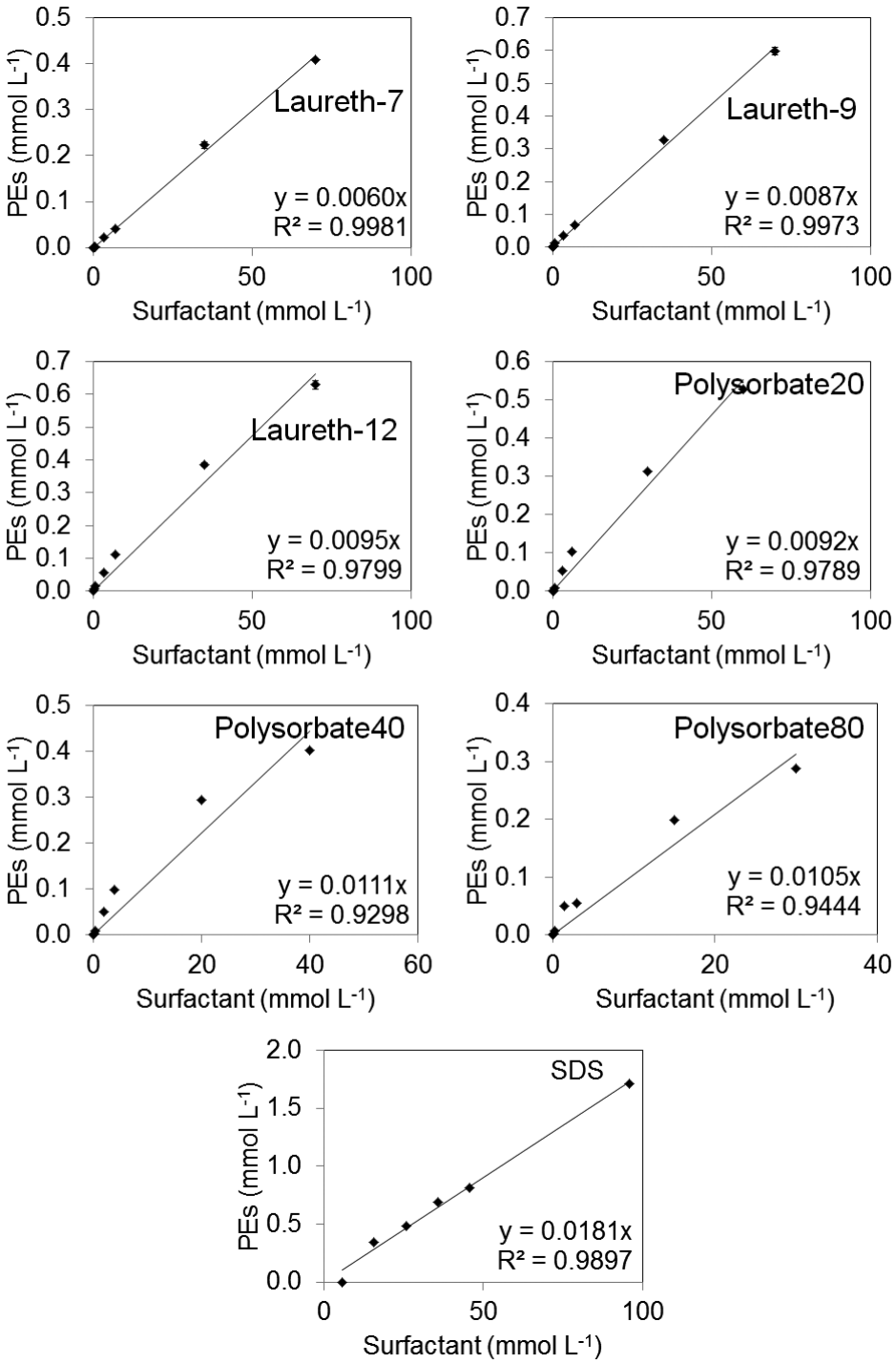

Figure a-2 MSR determination plot of nonionic surfactants

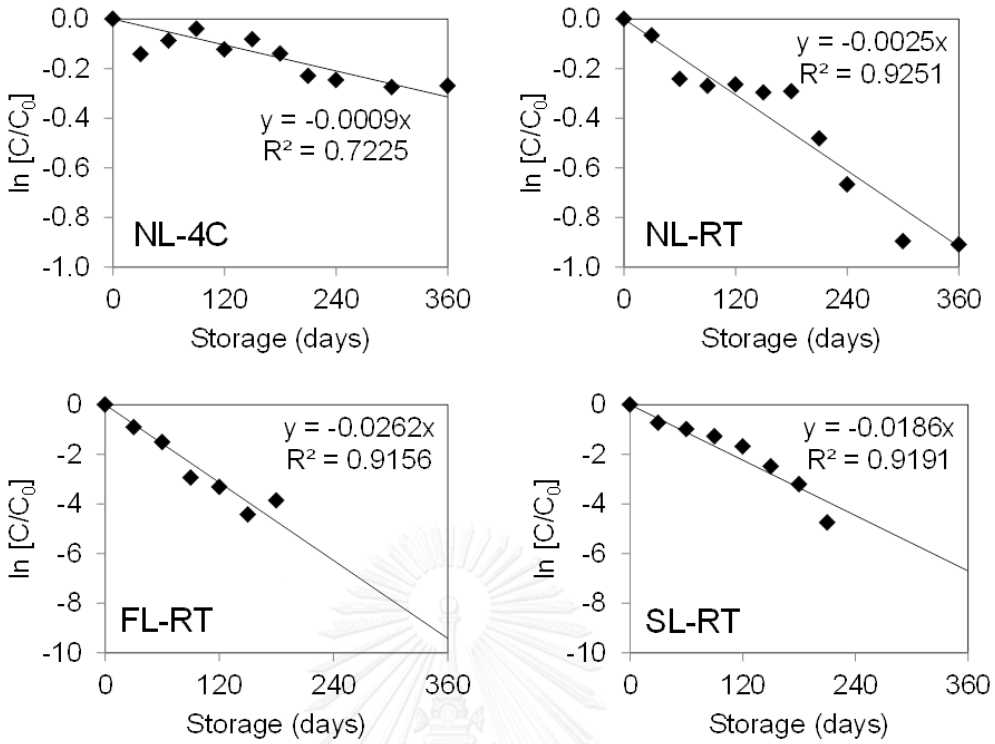

Figure a-3 Phorbol esters degradation over the oil storage time in the different storage conditions, the initial PEs in the Jatropha oil was 2.09 g L^{-1} as TPA equivalent. NL-4C: non-light exposure at 4°C, NL-RT: non-light exposure at room temperature, FL-RT: fluorescent light exposure at room temperature, and SL-RT: diffused sunlight at room temperature

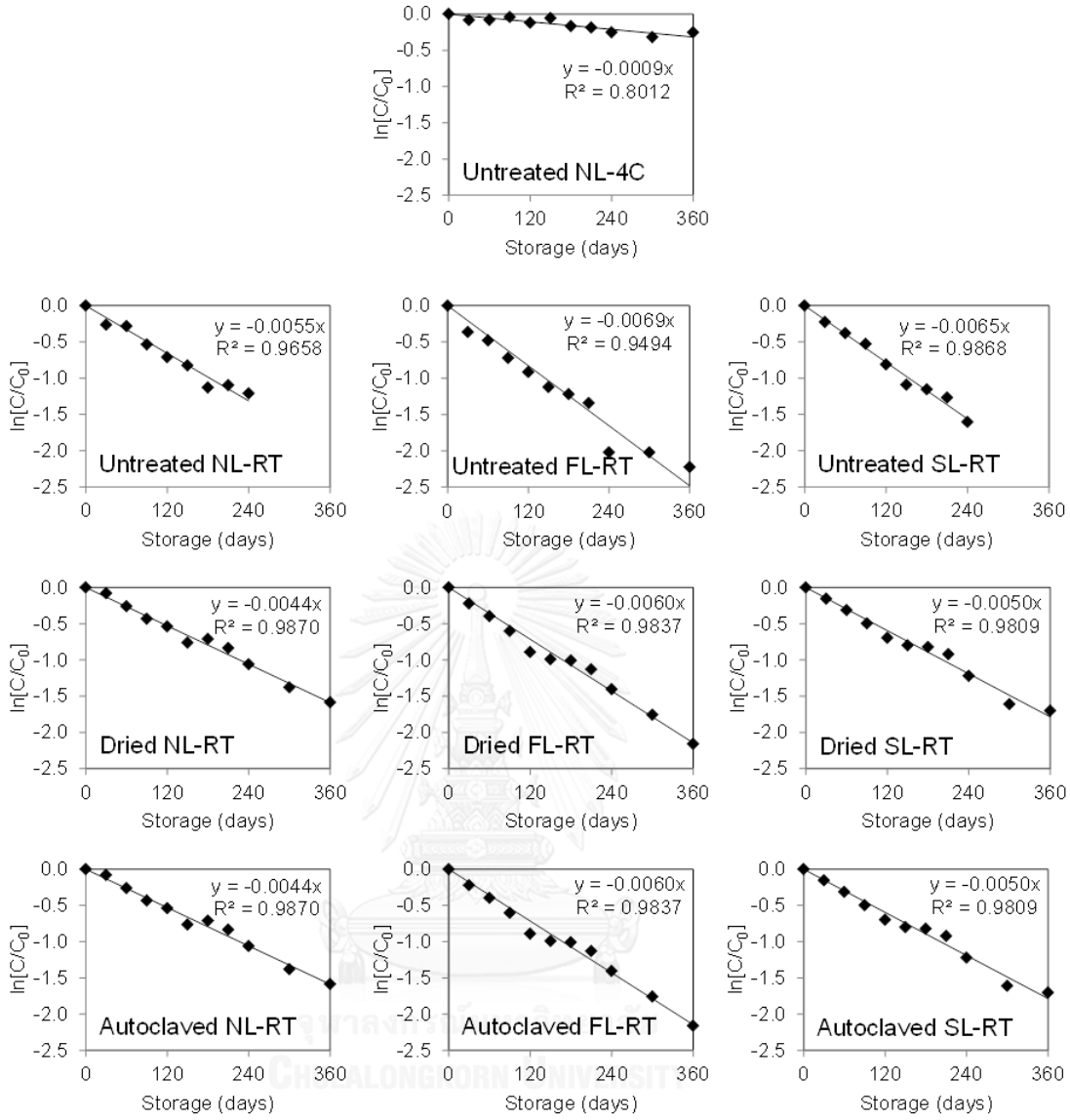

Figure a-4 Phorbol esters degradation over the Jatropha pressed seed storage time in the different storage conditions, the initial PEs in the untreated, dried, and autoclaved pressed seeds were (0.74, 0.76, 0.70) g kg^{-1} as TPA equivalent, respectively. NL-4C: non-light exposure at 4°C, NL-RT: non-light exposure at room temperature, FL-RT: fluorescent light exposure at room temperature, and SL-RT: diffused sunlight at room temperature

Appendix B One-way ANOVA in solubilization results

Appendix b-1 Carbon chain length effect on PEs solubilization in 20 mmol/L of nonionic surfactant solution

Descriptives: PEs solubilization (mmol/L)

C-chain	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower	Upper		
12	3	.3467	.01528	.00882	.3087	.3846	.33	.36
16	3	.4667	.06807	.03930	.2976	.6358	.39	.52
18	3	.4333	.03055	.01764	.3574	.5092	.40	.46
Total	9	.4156	.06579	.02193	.3650	.4661	.33	.52

ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.023	2	.012	5.954	.038
Within Groups	.012	6	.002		
Total	.035	8			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

Carbon chain lenght	N	Subset for alpha = 0.05	
		1	2
12	3	.3467	
18	3	.4333	.4333
16	3		.4667
Sig.		.052	.389
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

Appendix b-2 Ethylene oxide number effect on PEs solubilization in 20 mmol/L of nonionic surfactant solution

Descriptives: PEs solubilization (mmol/L)

EON	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
7	3	.2033	.02082	.01202	.1516	.2550	.18	.22
9	3	.2567	.02309	.01333	.1993	.3140	.23	.27
12	3	.3967	.03055	.01764	.3208	.4726	.37	.43
Total	9	.2856	.08918	.02973	.2170	.3541	.18	.43

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.060	2	.030	47.228	.000
Within Groups	.004	6	.001		
Total	.064	8			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

Ethylene oxide number	N	Subset for alpha = 0.05		
		1	2	3
7	3	.2033		
9	3		.2567	
12	3			.3967
Sig.		1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 3.000.				

Appendix b-3 Temperature effect on PEs and oil solubilization in 20 mmol/L of polyborbate 80 solution

Descriptives

Temperature (°C)		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	20	3	.3333	.02517	.01453	.2708	.3958	.31	.36
	40	3	.2800	.02646	.01528	.2143	.3457	.25	.30
	60	3	.2433	.02517	.01453	.1808	.3058	.22	.27
	Total	9	.2856	.04503	.01501	.2509	.3202	.22	.36
Oil solubilization (mmol/L)	20	3	.4133	.04509	.02603	.3013	.5253	.37	.46
	40	3	.7100	.09539	.05508	.4730	.9470	.65	.82
	60	3	1.1200	.11136	.06429	.8434	1.3966	1.02	1.24
	Total	9	.7478	.31673	.10558	.5043	.9912	.37	1.24

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	.012	2	.006	9.373	.014
	Within Groups	.004	6	.001		
	Total	.016	8			
Oil solubilization (mmol/L)	Between Groups	.755	2	.378	48.154	.000
	Within Groups	.047	6	.008		
	Total	.803	8			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

Temperature (°C)	N	Subset for alpha = 0.05	
		1	2
60	3	.2433	
40	3	.2800	
20	3		.3333
Sig.		.130	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Oil solubilization (mmol/L)

Temperature (°C)	N	Subset for alpha = 0.05		
		1	2	3
20	3	.4133		
40	3		.7100	
60	3			1.1200
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-4 Temperature effect on PEs and oil solubilization in 20 mmol/L of laureth-12 solution

Descriptives

Temperature (°C)		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	20	3	.2400	.04583	.02646	.1262	.3538	.19	.28
	40	3	.2300	.01000	.00577	.2052	.2548	.22	.24
	60	3	.2100	.01000	.00577	.1852	.2348	.20	.22
	Total	9	.2267	.02739	.00913	.2056	.2477	.19	.28
Oil solubilization (mmol/L)	20	3	.0100	.00000	.00000	.0100	.0100	.01	.01
	40	3	.0200	.01732	.01000	-.0230	.0630	.01	.04
	60	3	.0467	.00577	.00333	.0323	.0610	.04	.05
	Total	9	.0256	.01878	.00626	.0111	.0400	.01	.05

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	.001	2	.001	.913	.451
	Within Groups	.005	6	.001		
	Total	.006	8			
Oil solubilization (mmol/L)	Between Groups	.002	2	.001	9.700	.013
	Within Groups	.001	6	.000		
	Total	.003	8			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

Temperature (°C)	N	Subset for alpha = 0.05
		1
60	3	.2100
40	3	.2300
20	3	.2400
Sig.		.247

Oil solubilization (mmol/L)

Temperature (°C)	N	Subset for alpha = 0.05	
		1	2
20	3	.0100	
40	3	.0200	
60	3		.0467
Sig.		.289	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-5 Ethanol effect on PEs and oil solubilization in 20 mmol/L of polyborbate 80 solution

Descriptives

Ethanol additive (%v/v)		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	.0	3	.2933	.02517	.01453	.2308	.3558	.27	.32
	2.5	3	.3000	.01000	.00577	.2752	.3248	.29	.31
	5.0	3	.3167	.01155	.00667	.2880	.3454	.31	.33
	7.5	3	.3567	.04041	.02333	.2563	.4571	.32	.40
	10.0	3	.4300	.05292	.03055	.2986	.5614	.39	.49
	Total	15	.3393	.05898	.01523	.3067	.3720	.27	.49
Oil solubilization (mmol/L)	.0	3	.1933	.04933	.02848	.0708	.3159	.16	.25
	2.5	3	.1600	.01000	.00577	.1352	.1848	.15	.17
	5.0	3	.1533	.01528	.00882	.1154	.1913	.14	.17
	7.5	3	.1400	.01000	.00577	.1152	.1648	.13	.15
	10.0	3	.1567	.02309	.01333	.0993	.2140	.13	.17
	Total	15	.1607	.02865	.00740	.1448	.1765	.13	.25

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	.038	4	.010	8.984	.002
	Within Groups	.011	10	.001		
	Total	.049	14			
Oil solubilization (mmol/L)	Between Groups	.005	4	.001	1.725	.221
	Within Groups	.007	10	.001		
	Total	.011	14			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

Ethanol additive (%v/v)	N	Subset for alpha = 0.05	
		1	2
.0	3	.2933	
2.5	3	.3000	
5.0	3	.3167	
7.5	3	.3567	
10.0	3		.4300
Sig.		.050	1.000

Oil solubilization (mmol/L)

Ethanol additive (%v/v)	N	Subset for alpha = 0.05	
		1	2
7.5	3	.1400	
5.0	3	.1533	.1533
10.0	3	.1567	.1567
2.5	3	.1600	.1600
.0	3		.1933
Sig.		.401	.110

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-6 Ethanol effect on PEs and oil solubilization in 20 mmol/L of laureth-12 solution

Descriptives

Ethanol additive (%v/v)		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	.0	3	.2333	.00577	.00333	.2190	.2477	.23	.24
	2.5	3	.2600	.01732	.01000	.2170	.3030	.24	.27
	5.0	3	.2667	.01155	.00667	.2380	.2954	.26	.28
	7.5	3	.2867	.01155	.00667	.2580	.3154	.28	.30
	10.0	3	.3067	.02082	.01202	.2550	.3584	.29	.33
	Total	15	.2707	.02840	.00733	.2549	.2864	.23	.33
Oil solubilization (mmol/L)	.0	3	.0067	.00577	.00333	-.0077	.0210	.00	.01
	2.5	3	.0500	.05196	.03000	-.0791	.1791	.02	.11
	5.0	3	.0333	.02517	.01453	-.0292	.0958	.01	.06
	7.5	3	.0433	.02082	.01202	-.0084	.0950	.02	.06
	10.0	3	.0167	.02082	.01202	-.0350	.0684	.00	.04
	Total	15	.0300	.02976	.00768	.0135	.0465	.00	.11

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	.009	4	.002	11.161	.001
	Within Groups	.002	10	.000		
	Total	.011	14			
Oil solubilization (mmol/L)	Between Groups	.004	4	.001	1.161	.384
	Within Groups	.008	10	.001		
	Total	.012	14			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

Ethanol additive (%v/v)	N	Subset for alpha = 0.05		
		1	2	3
.0	3	.2333		
2.5	3		.2600	
5.0	3		.2667	
7.5	3		.2867	.2867
10.0	3			.3067
Sig.		1.000	.055	.119

Oil solubilization (mmol/L)

Ethanol additive (%v/v)	N	Subset for alpha = 0.05
		1
.0	3	.0067
10.0	3	.0167
5.0	3	.0333
7.5	3	.0433
2.5	3	.0500
Sig.		.124

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-7 NaCl effect on PEs solubilization in 20 mmol/L of SDS solution

Descriptives

NaCl (mmol/L)		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	.00	3	.5767	.03215	.01856	.4968	.6565	.54	.60
	10.00	3	.8433	.04041	.02333	.7429	.9437	.80	.88
	20.00	3	1.0600	.06557	.03786	.8971	1.2229	1.00	1.13
	50.00	3	1.3500	.06083	.03512	1.1989	1.5011	1.28	1.39
	100.00	3	1.3200	.06557	.03786	1.1571	1.4829	1.26	1.39
	200.00	3	.8833	.04933	.02848	.7608	1.0059	.85	.94
	Total	18	1.0056	.28407	.06696	.8643	1.1468	.54	1.39
Oil solubilization (mmol/L)	.00	3	.0800	.01000	.00577	.0552	.1048	.07	.09
	10.00	3	.1467	.01528	.00882	.1087	.1846	.13	.16
	20.00	3	.2533	.02309	.01333	.1960	.3107	.24	.28
	50.00	3	.8333	.09074	.05239	.6079	1.0587	.75	.93
	100.00	3	1.5767	.04509	.02603	1.4647	1.6887	1.53	1.62
	200.00	3	2.3800	.11269	.06506	2.1001	2.6599	2.31	2.51
	Total	18	.8783	.87352	.20589	.4439	1.3127	.07	2.51

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	1.337	5	.267	92.210	.000
	Within Groups	.035	12	.003		
	Total	1.372	17			
Oil solubilization (mmol/L)	Between Groups	12.924	5	2.585	650.718	.000
	Within Groups	.048	12	.004		
	Total	12.972	17			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

NaCl (mmol/L)	N	Subset for alpha = 0.05			
		1	2	3	4
.00	3	.5767			
10.00	3		.8433		
200.00	3		.8833		
20.00	3			1.06	
100.00	3				1.32
50.00	3				1.35
Sig.		1.000	.381	1.00	.508

Oil solubilization (mmol/L)

NaCl (mmol/L)	N	Subset for alpha = 0.05				
		1	2	3	4	5
.00	3	.080				
10.00	3	.146	.146			
20.00	3		.253			
50.00	3			.833		
100.00	3				1.576	
200.00	3					2.38
Sig.		.220	.060	1.00	1.0	1.0

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-8 Mix HLB of polysorbate 20:laureth-7 effect on PEs and oil solubilization in 20 mmol/L of total surfactant solution

Descriptives

HLB		N	Mean	Std. Deviation	Std. Error	95% C.I.		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	12.1	3	.1080	.00100	.00058	.1055	.1105	.11	.11
	14.1	3	.1147	.00058	.00033	.1132	.1161	.11	.12
	15.2	3	.1327	.00643	.00371	.1167	.1486	.13	.14
	15.9	3	.1493	.00513	.00296	.1366	.1621	.15	.16
	16.3	3	.1670	.00954	.00551	.1433	.1907	.16	.18
	Total	15	.1343	.02306	.00595	.1216	.1471	.11	.18
Oil solubilization (mmol/L)	12.1	3	.1657	.02743	.01584	.0975	.2338	.13	.18
	14.1	3	.0377	.00462	.00267	.0262	.0491	.04	.04
	15.2	3	.0293	.00208	.00120	.0242	.0345	.03	.03
	15.9	3	.0420	.01044	.00603	.0161	.0679	.04	.05
	16.3	3	.0780	.00361	.00208	.0690	.0870	.08	.08
	Total	15	.0705	.05338	.01378	.0410	.1001	.03	.18

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	.007	4	.002	55.667	.000
	Within Groups	.000	10	.000		
	Total	.007	14			
Oil solubilization (mmol/L)	Between Groups	.038	4	.010	52.908	.000
	Within Groups	.002	10	.000		
	Total	.040	14			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

HLB	N	Subset for alpha = 0.05			
		1	2	3	4
12.1	3	.1080			
14.1	3	.1147			
15.2	3		.1327		
15.9	3			.1493	
16.3	3				.1670
Sig.		.180	1.000	1.000	1.000

Oil solubilization (mmol/L)

HLB	N	Subset for alpha = 0.05		
		1	2	3
15.2	3	.0293		
14.1	3	.0377		
15.9	3	.0420		
16.3	3		.0780	
12.1	3			.1657
Sig.		.296	1.000	1.000

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-9 Mix HLB of polysorbate 20:laureth-9 effect

Descriptives

HLB		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	13.4	3	.1763	.01069	.00617	.1498	.2029	.16	.18
	14.7	3	.1597	.00252	.00145	.1534	.1659	.16	.16
	15.4	3	.1733	.00252	.00145	.1671	.1796	.17	.18
	16.0	3	.1720	.01493	.00862	.1349	.2091	.16	.19
	16.4	3	.1903	.00586	.00338	.1758	.2049	.19	.20
	16.7	3	.1990	.01044	.00603	.1731	.2249	.19	.21
	Total	18	.1784	.01525	.00359	.1709	.1860	.16	.21
Oil solubilization (mmol/L)	13.4	3	.1133	.00231	.00133	.1076	.1191	.11	.12
	14.7	3	.0457	.00666	.00384	.0291	.0622	.04	.05
	15.4	3	.0277	.00513	.00296	.0149	.0404	.02	.03
	16.0	3	.0567	.01380	.00797	.0224	.0909	.04	.07
	16.4	3	.0723	.00709	.00410	.0547	.0900	.07	.08
	16.7	3	.1103	.01343	.00775	.0770	.1437	.10	.12
	Total	18	.0710	.03360	.00792	.0543	.0877	.02	.12

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	.003	5	.001	7.214	.002
	Within Groups	.001	12	.000		
	Total	.004	17			
Oil solubilization (mmol/L)	Between Groups	.018	5	.004	43.939	.000
	Within Groups	.001	12	.000		
	Total	.019	17			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

HLB	N	Subset for alpha = 0.05		
		1	2	3
14.7	3	.1597		
16.0	3	.1720		
15.4	3	.1733		
13.4	3	.1763	.1763	
16.4	3		.1903	.1903
16.7	3			.1990
Sig.		.058	.083	.264

Oil solubilization (mmol/L)

HLB	N	Subset for alpha = 0.05			
		1	2	3	4
15.4	3	.0277			
14.7	3		.0457		
16.0	3		.0567	.0567	
16.4	3			.0723	
16.7	3				.1103
13.4	3				.1133
Sig.		1.000	.165	.057	.694

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-10 Mix HLB of polysorbate 20:laureth-12 effect

Descriptives

HLB		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	13.4	3	.2167	.00473	.00273	.2049	.2284	.21	.22
	14.5	3	.2093	.00208	.00120	.2042	.2145	.21	.21
	15.3	3	.1960	.00964	.00557	.1720	.2200	.19	.20
	15.9	3	.1993	.00611	.00353	.1842	.2145	.19	.21
	16.3	3	.1833	.00751	.00433	.1647	.2020	.18	.19
	16.7	3	.1990	.01044	.00603	.1731	.2249	.19	.21
	Total	18	.2006	.01241	.00293	.1944	.2068	.18	.22
Oil solubilization (mmol/L)	13.4	3	.0437	.00115	.00067	.0408	.0465	.04	.05
	14.5	3	.0330	.00557	.00321	.0192	.0468	.03	.04
	15.3	3	.0367	.00569	.00328	.0225	.0508	.03	.04
	15.9	3	.0583	.00306	.00176	.0507	.0659	.06	.06
	16.3	3	.0777	.00493	.00285	.0654	.0899	.07	.08
	16.7	3	.1103	.01343	.00775	.0770	.1437	.10	.12
	Total	18	.0599	.02841	.00670	.0458	.0741	.03	.12

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	.002	5	.000	7.347	.002
	Within Groups	.001	12	.000		
	Total	.003	17			
Oil solubilization (mmol/L)	Between Groups	.013	5	.003	56.694	.000
	Within Groups	.001	12	.000		
	Total	.014	17			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

HLB	N	Subset for alpha = 0.05		
		1	2	3
16.3	3	.1833		
15.3	3	.1960	.1960	
16.7	3		.1990	
15.9	3		.1993	
14.5	3		.2093	.2093
13.4	3			.2167
Sig.		.056	.061	.244

Oil solubilization (mmol/L)

HLB	N	Subset for alpha = 0.05			
		1	2	3	4
14.5	3	.0330			
15.3	3	.0367			
13.4	3	.0437			
15.9	3		.0583		
16.3	3			.0777	
16.7	3				.1103
Sig.		.092	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-11 Mix HLB of polysorbate 20: polysorbate 80 effect

Descriptives

HLB		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min.	Max.
						Lower	Upper		
PEs solubilization (mmol/L)	15.0	3	.1893	.03265	.01885	.1082	.2705	.17	.23
	15.3	3	.1790	.03759	.02170	.0856	.2724	.14	.22
	15.7	3	.2083	.04751	.02743	.0903	.3264	.18	.26
	16.0	3	.1863	.02050	.01184	.1354	.2373	.17	.21
	16.3	3	.2167	.03086	.01782	.1400	.2933	.20	.25
	16.7	3	.1990	.01044	.00603	.1731	.2249	.19	.21
	Total	18	.1964	.03017	.00711	.1814	.2114	.14	.26
Oil solubilization (mmol/L)	15.0	3	.1633	.01935	.01117	.1153	.2114	.14	.18
	15.3	3	.1413	.00643	.00371	.1254	.1573	.13	.15
	15.7	3	.1380	.03500	.02021	.0511	.2249	.10	.17
	16.0	3	.1300	.01453	.00839	.0939	.1661	.12	.15
	16.3	3	.1183	.01674	.00967	.0767	.1599	.10	.13
	16.7	3	.1103	.01343	.00775	.0770	.1437	.10	.12
	Total	18	.1336	.02410	.00568	.1216	.1455	.10	.18

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs solubilization (mmol/L)	Between Groups	.003	5	.001	.587	.710
	Within Groups	.012	12	.001		
	Total	.015	17			
Oil solubilization (mmol/L)	Between Groups	.005	5	.001	2.725	.072
	Within Groups	.005	12	.000		
	Total	.010	17			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs solubilization (mmol/L)

HLB	N	Subset for alpha = 0.05
		1
15.3	3	.1790
16.0	3	.1863
15.0	3	.1893
16.7	3	.1990
15.7	3	.2083
16.3	3	.2167
Sig.		.219

Oil solubilization (mmol/L)

HLB	N	Subset for alpha = 0.05	
		1	2
16.7	3	.1103	
16.3	3	.1183	
16.0	3	.1300	.1300
15.7	3	.1380	.1380
15.3	3	.1413	.1413
15.0	3		.1633
Sig.		.103	.077

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-12 SDS miix with laureth-7 effect on PEs solubilization in 20 mmol/L of total surfactant solution

Descriptives: PEs solubilization (mmol/L)

SDS (mmol/L)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower	Upper		
0	3	.2047	.01909	.01102	.1573	.2521	.18	.22
5	3	.2747	.00462	.00267	.2632	.2861	.27	.28
10	3	.3763	.02676	.01545	.3098	.4428	.35	.41
15	3	.5520	.02252	.01300	.4961	.6079	.53	.57
Total	12	.3519	.13753	.03970	.2645	.4393	.18	.57

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.205	3	.068	169.744	.000
Within Groups	.003	8	.000		
Total	.208	11			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

SDS concentration (mmol/L)	N	Subset for alpha = 0.05			
		1	2	3	4
0	3	.2047			
5	3		.2747		
10	3			.3763	
15	3				.5520
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-13 SDS miix with laureth-9 effect on PEs solubilization in 20 mmol/L of total surfactant solution

Descriptives: PEs solubilization (mmol/L)

SDS (mmol/L)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower	Upper		
0	3	.2540	.02524	.01457	.1913	.3167	.23	.27
5	3	.2847	.01210	.00698	.2546	.3147	.27	.29
10	3	.3363	.00586	.00338	.3218	.3509	.33	.34
15	3	.4447	.01604	.00926	.4048	.4845	.43	.46
Total	12	.3299	.07699	.02223	.2810	.3788	.23	.46

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.063	3	.021	78.213	.000
Within Groups	.002	8	.000		
Total	.065	11			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

SDS concentration (mmol/L)	N	Subset for alpha = 0.05		
		1	2	3
0	3	.2540		
5	3	.2847		
10	3		.3363	
15	3			.4447
Sig.		.051	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-14 SDS miix with laureth-12 effect on PEs solubilization in 20 mmol/L of total surfactant solution

Descriptives: PEs solubilization (mmol/L)

SDS (mmol/L)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower	Upper		
0	3	.3983	.02577	.01488	.3343	.4624	.38	.43
5	3	.4067	.01710	.00987	.3642	.4491	.39	.42
10	3	.4243	.01721	.00994	.3816	.4671	.41	.44
15	3	.4830	.01480	.00854	.4462	.5198	.47	.49
Total	12	.4281	.03822	.01103	.4038	.4524	.38	.49

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.013	3	.004	11.885	.003
Within Groups	.003	8	.000		
Total	.016	11			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

SDS concentration (mmol/L)	N	Subset for alpha = 0.05	
		1	2
0	3	.3983	
5	3	.4067	
10	3	.4243	
15	3		.4830
Sig.		.150	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-15 SDS miix with polysorbate 20 effect on PEs solubilization in 20 mmol/L of total surfactant solution

Descriptives: PEs solubilization (mmol/L)

SDS (mmol/L)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower	Upper		
0	3	.3483	.01419	.00819	.3131	.3836	.33	.36
5	3	.2933	.01007	.00581	.2683	.3183	.28	.30
10	3	.2927	.00379	.00219	.2833	.3021	.29	.30
15	3	.3273	.00987	.00570	.3028	.3518	.32	.33
Total	12	.3154	.02615	.00755	.2988	.3320	.28	.36

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.007	3	.002	21.536	.000
Within Groups	.001	8	.000		
Total	.008	11			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

SDS concentration (mmol/L)	N	Subset for alpha = 0.05		
		1	2	3
10	3	.2927		
5	3	.2933		
15	3		.3273	
0	3			.3483
Sig.		.938	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-16 SDS miix with polysorbate 40 effect on PEs solubilization in 20 mmol/L of total surfactant solution

Descriptives: PEs solubilization (mmol/L)

SDS (mmol/L)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower	Upper		
0	3	.4653	.06285	.03629	.3092	.6215	.40	.52
5	3	.4987	.03355	.01937	.4153	.5820	.46	.52
10	3	.3447	.13285	.07670	.0146	.6747	.26	.50
15	3	.4027	.00839	.00484	.3818	.4235	.39	.41
Total	12	.4278	.08919	.02575	.3712	.4845	.26	.52

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.042	3	.014	2.452	.138
Within Groups	.046	8	.006		
Total	.088	11			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

SDS concentration (mmol/L)	N	Subset for alpha = 0.05	
		1	2
10	3	.3447	
15	3	.4027	.4027
0	3	.4653	.4653
5	3		.4987
Sig.		.097	.174

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix b-17 SDS miix with polysorbate 80 effect on PEs solubilization in 20 mmol/L of total surfactant solution

Descriptives: PEs solubilization (mmol/L)

SDS (mmol/L)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower	Upper		
0	3	.4347	.03134	.01810	.3568	.5125	.40	.46
5	3	.4690	.05370	.03101	.3356	.6024	.44	.53
10	3	.4680	.04355	.02515	.3598	.5762	.43	.52
15	3	.4040	.01552	.00896	.3654	.4426	.39	.42
Total	12	.4439	.04335	.01251	.4164	.4715	.39	.53

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.009	3	.003	1.924	.204
Within Groups	.012	8	.002		
Total	.021	11			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

SDS concentration (mmol/L)	N	Subset for alpha = 0.05
		1
15	3	.4040
0	3	.4347
10	3	.4680
5	3	.4690
Sig.		.090
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

Appendix C One-way ANOVA in extraction results

Appendix c-1 EON effect on PEs extraction efficiency

Descriptives: PEs extraction efficiency (%)

EON	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower	Upper		
0	9	21.5578	11.90844	3.96948	12.4041	30.7114	9.84	40.10
2	6	36.8967	7.12337	2.90810	29.4212	44.3722	30.29	47.92
7	6	39.3567	2.22997	.91038	37.0165	41.6969	37.79	43.57
9	6	37.9100	2.12983	.86950	35.6749	40.1451	34.31	40.85
12	6	52.8283	3.46502	1.41459	49.1920	56.4646	48.61	58.28
20	12	56.0783	3.21062	.92683	54.0384	58.1183	51.77	61.68
Total	45	41.5313	14.09441	2.10107	37.2969	45.7658	9.84	61.68

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7131.542	5	1426.308	34.568	.000
Within Groups	1609.165	39	41.261		
Total	8740.707	44			

Post Hoc Tests, Homogeneous Subsets, Duncan[a,,b]

EON	N	Subset for alpha = 0.05		
		1	2	3
0	9	21.5578		
2	6		36.8967	
9	6		37.9100	
7	6		39.3567	
12	6			52.8283
20	12			56.0783
Sig.		1.000	.506	.351

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.968.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix c-2 Surfactant type effect on PEs extraction efficiency

Descriptives: PEs extraction efficiency (%)

Surfactant type	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower	Upper		
Nonionic	30	48.4503	8.70387	1.58910	45.2003	51.7004	34.31	61.68
Anionic	12	31.9792	10.14142	2.92758	25.5356	38.4227	10.84	47.92
No surfactant	3	10.5500	.94303	.54446	8.2074	12.8926	9.84	11.62
Total	45	41.5313	14.09441	2.10107	37.2969	45.7658	9.84	61.68

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5410.633	2	2705.316	34.120	.000
Within Groups	3330.075	42	79.287		
Total	8740.707	44			

Post Hoc Tests, Homogeneous Subsets, Duncan[a,b]

Surfactant type	N	Subset for alpha = 0.05		
		1	2	3
No surfactant	3	10.5500		
Anionic surfactant	12		31.9792	
Nonionic surfactant	30			48.4503
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.667.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix c-3 Surfactant solution effect on PEs extraction efficiency

Descriptives: PEs extraction efficiency (%)

Surfactant name	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower	Upper		
Laureth-7	6	39.3567	2.22997	.91038	37.0165	41.6969	37.79	43.57
Laureth-9	6	37.9100	2.12983	.86950	35.6749	40.1451	34.31	40.85
Laureth-12	6	52.8283	3.46502	1.41459	49.1920	56.4646	48.61	58.28
Polysorbate 20	6	54.5350	2.77621	1.13338	51.6215	57.4485	51.77	57.79
Polysorbate 80	6	57.6217	3.04202	1.24190	54.4293	60.8141	53.79	61.68
SDS	6	27.0617	10.83918	4.42508	15.6866	38.4367	10.84	40.10
SLES	6	36.8967	7.12337	2.90810	29.4212	44.3722	30.29	47.92
water	3	10.5500	.94303	.54446	8.2074	12.8926	9.84	11.62
Total	45	41.5313	14.09441	2.10107	37.2969	45.7658	9.84	61.68

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7705.395	7	1100.771	39.339	.000
Within Groups	1035.312	37	27.981		
Total	8740.707	44			

Post Hoc Tests, Homogeneous Subsets, Duncan[a,,b]

Surfactant name	N	Subset for alpha = 0.05			
		1	2	3	4
water	3	10.5500			
SDS	6		27.0617		
SLES	6			36.8967	
Laureth-9	6			37.9100	
Laureth-7	6			39.3567	
Laureth-12	6				52.8283
Polysorbate 20	6				54.5350
Polysorbate 80	6				57.6217
Sig.		1.000	1.000	.480	.171

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.333.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix c-4 Oil content effect on PEs extraction efficiency with 40 mmol/L of polysorbate 80 or laureth-12 solution

Descriptives: PEs extraction efficiency (%)

Oil content (%)		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
						Lower	Upper		
Polysorbate 80		3	61.967	.6028	.3480	60.469	63.464	61.4	62.6
	21.0	3	60.200	.8718	.5033	58.034	62.366	59.2	60.8
	25.2	3	58.300	1.1533	.6658	55.435	61.165	57.1	59.4
	29.3	3	54.333	2.2279	1.2863	48.799	59.868	52.9	56.9
	33.5	3	45.600	1.5395	.8888	41.776	49.424	44.3	47.3
	Total	15	56.080	6.1405	1.5855	52.679	59.481	44.3	62.6
Laureth-12	16.9	3	59.033	.3215	.1856	58.235	59.832	58.8	59.4
	21.0	3	63.167	1.5275	.8819	59.372	66.961	61.5	64.5
	25.2	3	64.100	.5292	.3055	62.786	65.414	63.7	64.7
	29.3	3	63.567	.4041	.2333	62.563	64.571	63.2	64.0
	33.5	3	57.867	1.7559	1.0138	53.505	62.229	56.2	59.7
	Total	15	61.547	2.8180	.7276	59.986	63.107	56.2	64.7

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Polysorbate 80	Between Groups	508.311	4	127.078	64.924	.000
	Within Groups	19.573	10	1.957		
	Total	527.884	14			
Laureth-12	Between Groups	99.251	4	24.813	20.804	.000
	Within Groups	11.927	10	1.193		
	Total	111.177	14			

Post Hoc Tests, Homogeneous Subset,s Duncan[a]

Polysorbate 80					
Oil content ($)	N	Subset for alpha = 0.05			
		1	2	3	4
33.5	3	45.60			
29.3	3		54.33		
25.2	3			58.30	
21.0	3			60.20	60.20
16.9	3				61.99
Sig.		1.000	1.000	.127	.153

Laureth-12			
Oil content (%)	N	Subset for alpha = 0.05	
		1	2
33.5	3	57.867	
16.9	3	59.033	
21.0	3		63.167
29.3	3		63.567
25.2	3		64.100
Sig.		.220	.341

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix c-5 Polysorbate 80 concentration effect on PEs extraction efficiency

Descriptives: PEs extraction efficiency (%)

Polysorbate 80 (mmol/L)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
10	3	40.4933	2.03230	1.17335	35.4448	45.5418	39.31	42.84
20	3	52.5967	2.63213	1.51966	46.0581	59.1353	50.39	55.51
40	3	60.0533	3.14786	1.81742	52.2336	67.8731	57.29	63.48
60	3	63.2067	2.33577	1.34856	57.4043	69.0090	60.54	64.89
80	3	63.1600	1.45564	.84042	59.5440	66.7760	61.68	64.59
100	3	64.3767	1.92056	1.10883	59.6057	69.1476	63.15	66.59
Total	18	57.3144	8.94058	2.10732	52.8684	61.7605	39.31	66.59

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1294.418	5	258.884	48.193	.000
Within Groups	64.461	12	5.372		
Total	1358.879	17			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

Polysorbate 80 concentration (mmol/L)	N	Subset for alpha = 0.05		
		1	2	3
10	3	40.4933		
20	3		52.5967	
40	3			60.0533
80	3			63.1600
60	3			63.2067
100	3			64.3767
Sig.		1.000	1.000	.055

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix c-6 Laureth-12 concentration effect on PEs extraction efficiency

Descriptives: PEs extraction efficiency (%)

Laureth-12 (mmol/L)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
10	3	28.0033	1.47846	.85359	24.3306	31.6760	26.72	29.62
20	3	52.4967	2.41086	1.39191	46.5078	58.4856	51.06	55.28
40	3	60.2700	1.29085	.74527	57.0633	63.4767	58.78	61.05
60	3	70.5400	1.16580	.67308	67.6440	73.4360	69.35	71.68
80	3	72.5633	1.27798	.73784	69.3887	75.7380	71.18	73.70
100	3	76.1233	1.33800	.77249	72.7996	79.4471	75.11	77.64
125	3	79.0700	4.70051	2.71384	67.3933	90.7467	75.65	84.43
175	3	80.9633	1.88089	1.08593	76.2910	85.6357	79.75	83.13
Total	24	65.0038	17.08774	3.48802	57.7882	72.2193	26.72	84.43

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6635.630	7	947.947	189.214	.000
Within Groups	80.159	16	5.010		
Total	6715.789	23			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

Concentration (mmol/L)	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
10	3	28.0033						
20	3		52.4967					
40	3			60.2700				
60	3				70.5400			
80	3				72.5633	72.5633		
100	3					76.1233	76.1233	
125	3						79.0700	79.0700
175	3							80.9633
Sig.		1.000	1.000	1.000	.285	.069	.126	.316

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix c-7 Extraction time effect on PEs extraction efficiency with laureth-12 solution at optimal condition with agitation

Descriptives: PE extraction efficiency (%)

Extract time (min)	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
5	3	69.6333	.37859	.21858	68.6929	70.5738	69.20	69.90
10	3	74.7667	1.65630	.95627	70.6522	78.8811	73.20	76.50
20	3	77.4333	.97125	.56075	75.0206	79.8461	76.60	78.50
30	3	79.3333	.30551	.17638	78.5744	80.0922	79.00	79.60
40	3	82.6333	1.60416	.92616	78.6484	86.6183	81.10	84.30
50	3	83.5667	2.25019	1.29915	77.9769	89.1564	81.30	85.80
Total	18	77.8944	5.01732	1.18259	75.3994	80.3895	69.20	85.80

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	404.829	5	80.966	42.024	.000
Within Groups	23.120	12	1.927		
Total	427.949	17			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

Extract time (min)	N	Subset for alpha = 0.05			
		1	2	3	4
5	3	69.6333			
10	3		74.7667		
20	3			77.4333	
30	3			79.3333	
40	3				82.6333
50	3				83.5667
Sig.		1.000	1.000	.119	.426

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix D One-way ANOVA in recovery results

Appendix d-1 Electrolyte additive comparison

Descriptives

Electrolytes		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
						Lower	Upper		
Rich phase volume (%)	NaCl	3	33.1667	1.40475	.81104	29.6771	36.6563	31.70	34.50
	Na_2CO_3	3	34.2333	2.92632	1.68951	26.9640	41.5027	31.00	36.70
	Na_2SO_4	3	34.5000	.00000	.00000	34.5000	34.5000	34.50	34.50
	Na_3PO_4	3	31.4333	1.69214	.97696	27.2298	35.6368	30.00	33.30
	Total	12	33.3333	2.00514	.57884	32.0593	34.6073	30.00	36.70
PEs recovery (%)	NaCl	3	88.2333	5.21664	3.01183	75.2745	101.1922	82.30	92.10
	Na_2CO_3	3	80.0333	2.21886	1.28106	74.5214	85.5453	78.00	82.40
	Na_2SO_4	3	91.4000	6.06053	3.49905	76.3448	106.4552	86.30	98.10
	Na_3PO_4	3	70.6333	2.91947	1.68556	63.3810	77.8857	68.80	74.00
	Total	12	82.5750	9.20308	2.65670	76.7276	88.4224	68.80	98.10
Laureth-12 recovery (%)	NaCl	3	93.8000	4.27200	2.46644	83.1878	104.4122	89.30	97.80
	Na_2CO_3	3	83.0667	4.02782	2.32546	73.0610	93.0723	80.40	87.70
	Na_2SO_4	3	87.1000	4.97594	2.87286	74.7391	99.4609	81.70	91.50
	Na_3PO_4	3	75.2333	3.33067	1.92296	66.9595	83.5072	71.50	77.90
	Total	12	84.8000	7.88036	2.27486	79.7931	89.8069	71.50	97.80

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
rich phase volume (%)	Between Groups	17.427	3	5.809	1.734	.237
	Within Groups	26.800	8	3.350		
	Total	44.227	11			
PEs recovery (%)	Between Groups	776.882	3	258.961	13.385	.002
	Within Groups	154.780	8	19.348		
	Total	931.662	11			
Laureth-12 recovery (%)	Between Groups	542.447	3	180.816	10.284	.004
	Within Groups	140.653	8	17.582		
	Total	683.100	11			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

rich phase volume (%)

Electrolytes	N	Subset for alpha = 0.05
		1
Na_3PO_4	3	31.4333
NaCl	3	33.1667
Na_2CO_3	3	34.2333
Na_2SO_4	3	34.5000
Sig.		.090

PEs recovery (%)

Electrolytes	N	Subset for alpha = 0.05		
		1	2	3
Na_3PO_4	3	70.6333		
Na_2CO_3	3		80.0333	
NaCl	3		88.2333	88.2333
Na_2SO_4	3			91.4000
Sig.		1.000	.052	.404

Laureth-12 recovery (%)

Electrolytes	N	Subset for alpha = 0.05		
		1	2	3
Na_3PO_4	3	75.2333		
Na_2CO_3	3	83.0667	83.0667	
Na_2SO_4	3		87.1000	87.1000
NaCl	3			93.8000
Sig.		.051	.273	.086

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix d-2 Comparison of fresh laureth-12 solution, reused solution, and methanol in PEs extraction process

Descriptives

Solutions		N	Mean	Std. Deviation	Std. Error	95% C.I.		Min.	Max.
						Lower	Upper		
PEs extraction efficiency (%)	9.4% laureth-12	3	81.76	1.12743	.65092	78.9593	84.5607	81.05	83.06
	9.4% laureth-12 + 5.9% Na2SO4	3	81.37	.99571	.57487	78.8999	83.8468	80.55	82.48
	9.4% laureth-12 reuse solution	3	74.40	.48993	.28286	73.1796	75.6137	74.07	74.96
	Methanol	3	80.96	.80525	.46491	78.9563	82.9570	80.14	81.75
	Total	12	79.62	3.25399	.93935	77.5542	81.6892	74.07	83.06
PEs concentration factor	9.4% laureth-12	3	4.34	.20599	.11893	3.8250	4.8484	4.12	4.53
	9.4% laureth-12 + 5.9% Na2SO4	3	4.04	.38760	.22378	3.0738	4.9995	3.60	4.34
	9.4% laureth-12 reuse solution	3	3.74	.507	.29294	2.4829	5.0037	3.21	4.22
	Methanol	3	21.13	.931	.53777	18.8161	23.4439	20.17	22.03
	Total	12	8.31	7.748	2.23675	3.3886	13.2347	3.21	22.03
Clude protein (g/ 100g)	9.4% laureth-12	2	25.16	.53033	.37500	20.4002	29.9298	24.79	25.54
	9.4% laureth-12 + 5.9% Na2SO4	2	19.24	.24042	.17000	17.0799	21.4001	19.07	19.41
	9.4% laureth-12 reuse solution	2	19.20	.65054	.46000	13.3551	25.0449	18.74	19.66
	Methanol	2	32.14	.55154	.39000	27.1846	37.0954	31.75	32.53
	Total	8	23.94	5.70292	2.01629	19.1685	28.7040	18.74	32.53

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PEs extraction efficiency (%)	Between Groups	110.171	3	36.724	46.618	.000
	Within Groups	6.302	8	.788		
	Total	116.473	11			
PEs concentration factor	Between Groups	657.767	3	219.256	665.571	.000
	Within Groups	2.635	8	.329		
	Total	660.402	11			
Clude protein (g/ 100g)	Between Groups	226.596	3	75.532	283.303	.000
	Within Groups	1.066	4	.267		
	Total	227.663	7			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

PEs extraction efficiency (%)

Solutions	N	Subset for alpha = 0.05	
		1	2
9.4% laureth-12 reuse solution	3	74.3967	
Methanol	3		80.9567
9.4% laureth-12 + 5.9% Na2SO4	3		81.3733
9.4% laureth-12	3		81.7600
Sig.		1.000	.319

PEs concentration factor

Solution	N	Subset for alpha = 0.05	
		1	2
9.4% laureth-12 reuse solution	3	3.7433	
9.4% laureth-12 + 5.9% Na2SO4	3	4.0367	
9.4% laureth-12	3	4.3367	
Methanol	3		21.1300
Sig.		.259	1.000

Clude protein (g/ 100g)

Solution	N	Subset for alpha = 0.05		
		1	2	3
9.4% laureth-12 reuse solution	2	19.2000		
9.4% laureth-12 + 5.9% Na2SO4	2	19.2400		
9.4% laureth-12	2		25.1650	
Methanol	2			32.1400
Sig.		.942	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix E One-way ANOVA in degradation rate results

Descriptives: k (10^{-3}/day)

Condition	N	Mean	Std. Deviation	Std. Error	95% C.I.		Min.	Max.
					Lower	Upper		
Oil - NL-4C	3	.8733	.00577	.00333	.8590	.8877	.87	.88
Oil - NL-RT	3	2.5333	.05774	.03333	2.3899	2.6768	2.50	2.60
Oil - FL-RT	3	26.1667	2.26789	1.30937	20.5329	31.8004	23.60	27.90
Oil - SL-RT	3	18.6333	2.40069	1.38604	12.6697	24.5970	17.10	21.40
JPS - NL-4C	3	.8800	.07810	.04509	.6860	1.0740	.83	.97
JPS - NL-RT	3	5.5000	.26458	.15275	4.8428	6.1572	5.30	5.80
JPS - FL-RT	3	6.8667	.23094	.13333	6.2930	7.4404	6.60	7.00
JPS - SL-RT	3	6.5333	.32146	.18559	5.7348	7.3319	6.30	6.90
Dried JPS - NL-RT	3	4.5333	.11547	.06667	4.2465	4.8202	4.40	4.60
Dried JPS - FL-RT	3	6.4667	.11547	.06667	6.1798	6.7535	6.40	6.60
Dried JPS - SL-RT	3	4.9333	.15275	.08819	4.5539	5.3128	4.80	5.10
Autoclaved JPS - NL-RT	3	4.4100	.01000	.00577	4.3852	4.4348	4.40	4.42
Autoclaved JPS - FL-RT	3	5.9667	.30551	.17638	5.2078	6.7256	5.70	6.30
Autoclaved JPS - SL-RT	3	4.9333	.15275	.08819	4.5539	5.3128	4.80	5.10
Total	42	7.0879	6.79082	1.04785	4.9717	9.2040	.83	27.90

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1868.105	13	143.700	177.885	.000
Within Groups	22.619	28	.808		
Total	1890.724	41			

Post Hoc Tests, Homogeneous Subsets, Duncan[a]

Condition	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
Oil - NL-4C	3	.87						
JPS - NL-4C	3	.88						
Oil - NL-RT	3		2.53					
Autoclaved JPS - NL-RT	3			4.41				
Dried JPS - NL-RT	3			4.53				
Dried JPS - SL-RT	3			4.93	4.93			
Autoclaved JPS - SL-RT	3			4.93	4.93			
JPS - NL-RT	3			5.50	5.50	5.50		
Autoclaved JPS - FL-RT	3			5.97	5.97	5.97		
Dried JPS - FL-RT	3				6.47	6.47		
JPS - SL-RT	3				6.53	6.53		
JPS - FL-RT	3					6.87		
Oil - SL-RT	3						18.637	
Oil - FL-RT	3							26.17
Sig.		.993	1.000	.070	.063	.106	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

VITA

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