# Study on Extraction, Quality and Application of Virgin Coconut Oil

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## บทคัดย่อ

ปัจจุบันการใช้น้ำมันมะพร้าวบริสุทธิ์ (virgin coconut oil, VCO) ในผลิตภัณฑ์อาหารได้รับ ความสนใจ เนื่องจากอุดมไปด้วยกรดไขมันขนาดกลาง (medium chain fatty acids, MCFAs) ที่ ง่ายต่อกระบวนการย่อยในร่างกาย และสารออกฤทธิ์ทางชีวภาพอื่น วิธีการสกัดน้ำมันส่งผลต่อ ปริมาณและคุณภาพของ VCO ที่ผลิตได้ จากการศึกษาการเตรียม VCO โดยเปรียบเทียบวิธีการสกัด น้ำมันแบบดั้งเดิม (การหมัก และการแช่แข็ง-ทำละลาย) กับวิธีที่ใช้เอนไซม์โปรติเอส พบว่าการสกัด ด้วยเอนไซม์ให้ปริมาณน้ำมันสูงที่สุด (P<0.05) รองลงมาคือการแช่แข็ง-ทำละลาย และการหมัก โดย ได้ผลการสกัดสูงสุดในแต่ละวิธีคิดเป็นร้อยละ 93.54, 79.00 และ74.93 ตามลำดับ กรดลอริคเป็น กรดไขมันหลักที่พบใน VCO ที่สกัดได้ โดยมีปริมาณในช่วงร้อยละ 49.51 ถึง 51.72 ของกรดไขมัน ทั้งหมด VCO ที่ได้จากการสกัดด้วยเอนไซม์โปรติเอสมีปริมาณกรดไขมันไม่อิ่มตัวสูงที่สุด ในขณะที่ การสกัดน้ำมันด้วยวิธีการหมักมีสารออกฤทธิ์ทางชีวภาพ ได้แก่ โทโคฟีรอลและสารประกอบฟีนอลิค ในปริมาณสูงที่สุด โดยสารฟีนอลิคหลักที่พบใน VCO ได้แก่ แคเทชิน กรดแกลลิก กรดวานิลิก และ กรดพารา-คูมาริก สมบัติทางกายภาพและเคมีของ VCO ที่ได้จากการศึกษาครั้งนี้เป็นไปตาม ข้อกำหนดของ Asian pacific coconut community บ่งบอกถึงการนำไปใช้ได้ในเชิงพาณิชย์ของ น้ำมันที่สกัดได้ เมื่อนำมะพร้าวที่มีอายุแตกต่างกัน 3 ช่วงอายุ ได้แก่ 11 เดือน (ช่วงอ่อน) 12 เดือน (ช่วงปานกลาง) และ 13 เดือน (ช่วงแก่) มาสกัดน้ำมันด้วยวิธีการใช้เอนไซม์โปรติเอส พบว่ามะพร้าว ช่วงปานกลางและช่วงแก่ให้ปริมาณน้ำมันมากกว่ามะพร้าวช่วงอ่อนอย่างมีนัยสำคัญ (P<0.05) อย่างไรก็ตาม VCO ที่ได้จากมะพร้าวช่วงอ่อนมีคุณภาพที่ดีบ่งชี้จากปริมาณกรดไขมันอิสระต่ำกว่า รวมถึงปริมาณกรดไขมันไม่อิ่มตัว โทโคฟีรอล และสารประกอบฟีนอลิคที่สูงที่สุด (P<0.05)

จากนั้นศึกษาการนำ VCO ที่ได้ไปใช้ในผลิตภัณฑ์อาหาร โดยเลือกระบบอิมัลชันแบบน้ำมัน ในน้ำ (oil-in-water emulsion, O/W) เป็นแบบจำลอง เตรียมตัวอย่างอิมัลชันจาก VCO ที่ปริมาณ น้ำมันต่างกัน (ร้อยละ 5 ถึง 40 โดยปริมาตร) ก่อนทำการศึกษาความคงตัวทางเคมีกายภาพที่ ระยะเวลาการเก็บรักษาต่างๆ (0 ถึง 4 สัปดาห์) เปรียบเทียบกับตัวอย่าง O/W ของน้ำมันถั่วเหลือง (soybean oil, SBO) จากการศึกษาพบว่าปริมาณน้ำมันและอายุการเก็บรักษาที่เพิ่มขึ้นส่งผลลด

ความเสถียรทางเคมีและกายภาพของตัวอย่าง โดย VCO อิมัลชันมีความคงตัวทางกายภาพและเคมีที่
ดีกว่า SBO อิมัลชัน โดยเฉพาะอย่างยิ่งเมื่อระบบมีปริมาณน้ำมันสูง

      การศึกษาในส่วนต่อไปคือการนำ VCO ไปใช้เตรียมน้ำสลัด โดยศึกษาร่วมกับการใช้
มอลโตเด็กซ์ตรินจากมันสำปะหลัง (tapioca maltodextrins, TMD) ที่ระดับความเข้มข้นต่าง ๆ
(ร้อยละ 0 ถึง 3 โดยน้ำหนัก) เป็นสารเพิ่มความคงตัว จากการศึกษาพบว่า TMD สามารถช่วยพัฒนา
ความคงตัวของน้ำสลัดได้โดยเฉพาะเมื่อความเข้มข้นของ TMD สูงขึ้น การเติม TMD ร้อยละ 3 ส่งผล
ให้อัตราการเพิ่มขนาดของหยดน้ำมัน อัตราการเกิดครีม และการเปลี่ยนแปลงค่าสี มีค่าน้อยที่สุด
ตลอดระยะเวลาการเก็บรักษา 8 สัปดาห์ (P<0.05) แนวโน้มการพัฒนาความคงตัวของน้ำสลัดจาก
การเติม TMD สอดคล้องกับการเพิ่มค่าความหนืดของระบบ ชนิดน้ำมันมีบทบาทสำคัญต่อระดับการ
เกิดลิพิดออกซิเดชัน โดยน้ำสลัดที่เตรียมจาก VCO มีเสถียรภาพต้านการเกิดออกซิเดชันที่ดีกว่า
ตัวอย่างจาก SBO สันนิษฐานว่าเป็นผลจากการที่ VCO มีปริมาณกรดไขมันอิ่มตัวและสารประกอบ
ฟีนอลิคสูงกว่า SBO การประเมินผลทางประสาทสัมผัสพบว่าน้ำสลัดที่เตรียมจาก VCO มีคะแนนการ
ยอมรับที่ดี โดยเฉพาะอย่างยิ่งเมื่อมีการเติม TMD ที่ระดับความเข้มข้นร้อยละ 3 จากการศึกษาแสดง
ให้เห็นว่าการใช้ VCO ร่วมกับการเติม TMD ในการเตรียมน้ำสลัดทำให้ได้ผลิตภัณฑ์ที่มีความคงตัว
ทางเคมีกายภาพ และการยอมรับทางประสาทสัมผัสที่ดี

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## ABSTRACT

Recently, there is growing popularity of virgin coconut oil (VCO) in food products because its abundant presence in medium chain fatty acids (MCFAs) with a good digestibility, and other bioactive compounds. Oil extraction technique played a crucial role on recovery efficiency and quality of the derived oil. In this work, the effect of oil extraction using conventional techniques (*i.e.* fermentation and thermal cycling methods) and protease aided means to prepare VCO was compared. The highest oil recovery yield was provided by enzyme aided extraction ($P<0.05$), followed by thermal cycling and fermentation techniques with the oil recovery yields of 93.54 %, 79.00 %, and 74.93 %, respectively. Lauric acid was predominantly found in all VCOs with the content ranging from 49.51 to 51.72 % of total fatty acids. Enzyme aided extraction provided VCO with the greatest amount of unsaturated fatty acids, whereas the highest contents of bioactive compounds involving total tocopherols and phenolics were found for the oil recovered through fermentation means. The majority phenolic compounds present in the VCO were catechin, gallic, vanillic and *p*-coumaric acids. Chemical properties of the VCOs produced in this work conformed to the standard of the Asian Pacific Coconut Community suggesting to a potential of the VCO to be available commercially. Then, the coconut fruits with different maturities, including 11 months old (young stage), 12 months old (intermediate stage), and 13 months old (old stage), were used to produce VCO via the protease aided extraction. It was found that the coconuts at intermediate– and old stages contained significantly higher oil content than the young stage ($P<0.05$). Nonetheless, the VCO recovered from young fruits possessed a better initial quality as indicated by lower free fatty acid content and higher amount of unsaturated fatty acids involving total tocopherols and phenolic compounds.

Next, utilization of VCO was studied by selecting oil-in-water emulsion (O/W) as a model. The emulsions were prepared using VCO at various oil contents (5–40 %, v/v), before observing their physicochemical stability at different storage times (0–4 weeks). The properties of VCO emulsions were compared with the counterparts made from soybean oil (SBO). By increasing oil content and storage time, colloidal and oxidative stabilities of the emulsions became decreased. Better physicochemical stability was evident for the VCO– compared to the SBO emulsions, especially with increased oil contents.

The further work was a preparation of salad dressings using VCO as a dispersed phase and Tapioca maltodextrin (TMD) at various concentrations (0–3 %, w/w) as a stabilizer. Improvement on stability of the dressings could be achieved by adding TMD, especially with the increased TMD concentration. Incorporation of TMD at 3 % led to the lowest oil droplet size increasing, creaming rate, and change in color of dressings along a storage of 8 weeks ($P<0.05$). Development on stability of the TMD added dressings was coincident with increased viscosity. Type of oil employed as a dispersed phase markedly influenced to lipid oxidative stability of dressings, in which the VCO dressings obviously showed better oxidative stability compared to the SBO counterparts. This could be postulated due to higher saturation degree of composited fatty acids and greater phenolic amounts of VCO than did SBO. Acceptable sensory score was reported for the VCO dressings in a presence of 3 % TMD. The present work suggests that VCO in incorporation with TMD could be used to produce salad dressings with desirable physicochemical stability and sensorial acceptance.

# ACKNOWLEDGEMENT

Finally, this study could not be successful without the financial support from the Graduate School, Prince of Songkla University.

# CONTENTS

**CONTENTS (Continued)**

**CONTENTS (Continued)**

# LIST OF TABLES

**LIST OF FIGURES**

## CHAPTER 1

## INTRODUCTION AND REVIEW OF LITERATURE

### 1.1 Introduction

Coconut oil (CO) is growing in popularity to be used as a functional ingredient for food processing, regarded to its richness in medium chain fatty acids (MCFAs) with a good digestibility (Che Man and Marina, 2006). Traditionally, CO is recovered by dry process using mechanical force to treat copra. The oil derived from this procedure has to be further treated through a refining, bleaching, and deodorizing (RBD) process. In RBD process, oil is always exposed to high temperature of *ca*. 204–245 °C that affects to increase free fatty acid and moisture contents, thereby leading to impair oil quality (O'Brien, 2004). By dry process, inferior oil quality also involves to contamination of aflatoxin in copra and cake (Guarte *et al.,* 1996). Recently, there is a trend towards producing CO by omitting RBD process. Wet extraction implemented by separating cream from fresh coconut meat and consequently breaking the milk emulsion is a promising way to recover CO with appreciable quality. By using wet extraction, chemical and heat treatment can be omitted from oil processing, so the oil recovered through this method is always defined as virgin coconut oil (VCO) (Villarino *et al.,* 2007).

Naturally, coconut milk is present in a form of oil-in-water (O/W) emulsion stabilized by coconut protein and phospholipids (Gopala *et al.,* 2010). To produce VCO, coconut milk emulsion has to be destabilized to release oil. Fermentation and thermal cycling methods are traditionally conducted to produce VCO. Enzymatic aided extraction is an emerging technology for oil recovering by offering many advantages compared to conventional means, such as eliminate solvent consumption and provide effective oil recovery yield. By using enzymes, good quality of oil could be provided in term of low free fatty acid content (Marina *et al.,* 2009a). Moreover, enzymatic extraction is always carried out under mild temperature and omitted solvent condition, making it as eco-friendly process (Fullbrook, 1983).

Various factors involving intrinsic (*i.e.*, cultivars and age of fruit) and extrinsic (*i.e*., harvesting time and growth location) factors could affect to characteristics of the recovered oil (Balleza and Sierra, 1976; Laureles *et al*., 2000; Santoso *et al.,* 1996).

Generally, coconut fruit with the age of 10 to 12 months is suitable for harvesting, because of the highest oil content (Balleza and Sierra, 1976). With different maturity stages, physiology and enzyme activity in plant cells were differed, thereby affecting to the amount and profile of several health promoting bioactive compounds, such as tocopherol, phenolics, squalene, and pigments of the extracted oils (Gutiérrez *et al.,* 1999; Ryan *et al.,* 2002; Baccouri *et al.,* 2008). In a presence of different constituents, characteristics of oil is influenced, thereby affecting to its further utilization and stability.

Oil containing food is abundantly present in a form of emulsion. Stability against phase separation and oxidative reaction is important to assure consumer's acceptability in emulsified product. With high amount of MCFAs, VCO is more polarity than other edible seed oils, and might exhibit interfacial activity that led to improve emulsion formability (Nor Hayati *et al.,* 2007). Due to saturated degree of MCFAs and presence of natural antioxidative compounds, moreover, VCO might possess good oxidative stability. In this study, utilization of VCO in a model emulsion and emulsified food product was studied. Salad dressing was selected as a food emulsion model regarded due to its popularity. Salad dressing always contains a large amount of oil (*ca.* more than 30 %), making it is prone to oxidative deterioration. Considering on a colloidal stability of the dressing model, tapioca maltodextrin (TMD) was employed as a stabilizer and its effect on the stability of dressing samples was also elucidated.

In the present work, effects of enzyme aided extraction on a production of VCO was investigated comparing with conventional method, *i.e.*, fermentation and freeze-thaw cycling, in order to establish effective condition to recover VCO with high yield and good quality. After that, influence of coconut fruit maturity on chemical properties of the recovered VCO was investigated. Next, utilization of VCO in a model O/W emulsion was studied by comparing with soybean oil (SBO). SBO was selected as comparative oil, because it is all purpose oil widely used in both industrial and household levels. The physicochemical stability of the model emulsions made from the selected oils was evaluated. Finally, VCO was employed to prepare salad dressing. The stability and physicochemical characteristics of the dressings were examined in parallel with the dressings made from SBO. In this part, TMD was

employed as stabilizer, and its concentration effect on properties of the dressings was also elucidated.

## 1.2 Review of literature

### 1.2.1 Coconut oil (CO)

CO is an edible oil widely used in tropical countries for thousands years in many industries including food, pharmaceuticals, and cosmetics. CO provides several advantages, such as richness in medium chain fatty acids (MCFAs) with a good digestibility and antiviral activity (Che Man and Marina, 2006; Marina *et al.,* 2009a). Coconut is very versatile and unique plant and bears fruit all year round.  It is very resilient and can withstand any type of weather or natural calamities. Conventionally, CO is produced by crushing copra, the dried kernel containing about 60–65 % oil content. CO has a natural sweet taste of coconut and contains most of saturated fatty acids (SFAs) of *ca.* 92 % of total fatty acids (TFAs) in a form of triglycerides. The predominant fatty acids (*ca.* 70 % TFAs) of CO are MCFAs, especially for lauric acid with the content of 45–56 % TFAs. Fatty acid composition of CO is revealed in **Table 1**. CO is characterized by a low iodine value, high saponification value and high SFAs content than those other vegetable oils. MCFAs are not commonly found in other vegetable oils. With their higher polarity than long chain fatty acids, MCFAs may act as emulsifier and solvent for flavors and some bioactive compounds (Nor Hayati *et al.,* 2007). Due to its richness in SFAs, CO is highly stable against lipid oxidation compared to other vegetable oils (Che Man and Marina, 2006; Marina *et al.,* 2009a). In term of nutritional value, MCFAs are easily metabolized without use of the carnitine transport system (Thampan, 1998), making CO as an appropriate energy source for some groups of people such as infant, pregnant and AIDs patients (Marina *et al.,* 2009a).

CO is present as a liquid at room temperature and insoluble in water. At temperature above its melting point, CO is completely miscible with most of the non-hydroxylic solvents such as light petroleum, benzene, and carbon tetrachloride. CO is more soluble in alcohol than most common fats and oils. CO contains some unsaponified constituents, involving tocopherols and phytosterols.

**Table 1** Fatty acid compositions of CO

| Fatty acids | % TFAs |
|---|---|
| Caprylic (C8:0) | $6.21 \pm 0.34$ |
| Capric (C10:0) | $6.15 \pm 0.21$ |
| Lauric (C12:0) | $51.02 \pm 0.71$ |
| Myristic (C14:0) | $18.94 \pm 0.63$ |
| Palmitic (C16:0) | $8.62 \pm 0.50$ |
| Stearic (C18:0) | $1.94 \pm 0.17$ |
| Oleic (C18:1) | $5.84 \pm 0.50$ |
| Linoleic (C18:2) | $1.28 \pm 0.18$ |

Source: Chowdhury *et al.* (2007)

Physicochemical properties of CO are important factors determining characteristic and stability of the oil. **Table 2** reveals some physicochemical properties of commercial CO based on Codex standard.

**1.2.2 Extraction of CO**

Conventionally, CO is produced by dry and solvent extraction methods. Firstly, fresh coconut meat has to be dried at *ca.* 40–50 ℃ for 30–45 min to receive copra, which is then further treated to recover oil through various techniques involving:

**1.2.2.1 Pressing**: Hydraulic press and screw presses are generally employed to produce CO. By using pressing means, low oil recovery yield is always provided.

**Table 2** Physicochemical properties of commercial CO based on Codex standard

| Characteristic | Value |
|---|---|
| Color-Platinum cobalt scale (max) | 50 |
| Relative density at 40 °C/20 °C (g/cm$^3$) | 0.908-0.921 |
| Refractive Index at 40 °C (°Brix) | 1.448-1.450 |
| Moisture & other volatiles at 105 °C (%) | 0.1 |
| Free fatty acids, calculated as lauric acid % by mass (max) | 0.3 |
| Peroxide value (millequivalents of active oxygen per kg) | Not more than 15 |
| Iodine value (g I$_2$/100g fat) | 6.3-10.6 |
| Sap. Value (mg KOH/g fat) | 248-265 |
| Unsaponifiables, % by mass, max g/kg | ≤ 15 |
| Reichert value (ml KOH/g fat) | 6-8.5 |
| Polenske value (ml C/g fat) | 13-18 |

Source: Codex Alimentarius (2001)

**1.2.2.2 Solvent extraction**: Solvent extraction is defined as a process that transporting materials from one phase to another for separating one or more compounds from mixtures. Hexane is the most used solvent for oil extraction. Solvent extraction provides high oil recovery yield than pressing method. However, it possesses high production cost. Several catastrophic explosions and fires of solvents residues are also bottleneck of this method.

CO derived by pressing and solvent extraction methods cannot be consumed directly, because it always contains solvent residues, dirt, microbial, spores, and other substances that may be harmful for consumer health. Therefore, the oil has to be further treated by refining, bleaching and deodorizing (RBD) process. In RBD process, the crude oil is firstly filtered, and then bleached using calcareous clays. After that, the oil is exposed to a very high temperature to deodorize and

destroy germs or fungal spores. Caustic soda is then introduced to remove mono or free fats. Finally, in some cases, the oil is hydrogenated to reduce unsaturated fatty acids content to extend shelf-life. RBD process affects to impair oil quality in several aspects, involving provide high free fatty acid content and loss of bioactive compounds and natural odor.

### 1.2.3 Virgin coconut oil (VCO)

VCO is defined as the oil obtained from fresh and mature kernel of coconut and recovered through mechanical or natural means with (not more than 60 ℃) or without heat application. VCO can be consumed in its natural state without RBD treatment. VCO is colorless, free of sediment with natural fresh coconut scent, and free from rancid odor or taste (Asian and Pacific Coconut Community (APCC), 2003). **Table 3** shows characteristics and quality factors of VCO according to the standard of APCC (2003).

VCO provides many advantages than CO in term of sensory quality and nutritional aspects. The difference between these two kinds of oil includes:

- *Source of the oil:* VCO is made from fresh coconut (mature coconut), while CO is produced using dried coconut or copra.

- *Extraction process:* This is a key differentiating factor between CO and VCO. VCO is exposed to heat, pressure and chemicals as least as possible, whereas chemical solvents and heat are always conducted in RBD process to produce CO.

- *Appearance:* Generally, both CO and VCO look almost the same in liquid form. Nevertheless, fine solid particles might be present in VCO, especially for the oil recovered by fermentation means.

- *Taste, smell and aroma:* This is an important key to distinguish VCO and CO. VCO tastes and smells like a fresh coconut, whereas RBD treated CO has a smell like other vegetable oils with no any natural taste and odor.

- *Smoking point:* Smoking point is the temperature that the oil starts to degrade when exposed to extreme temperature condition. Higher the smoking point, safer the oil for frying purpose. Smoking points of VCO and CO are close to 170 ℃ and above 220 ℃, respectively.

*- Other health promoting microconstituents:* Vitamin E and polyphenols are sensitive to heat and chemicals used in RBD process. Therefore, VCO shows better nutritional value than RBD treated ones (Onsaard *et al.,* 2006).

**Table 3** Characteristics and quality factors of VCO

| Parameters | value |
| --- | --- |
| Moisture (%) | Max 0.1 |
| Matters volatile at 120 ℃ (%) | Max 0.2 |
| Free fatty acid (%) | Max 0.2 |
| Peroxide value meq/kg | Max 3 |
| Relative density | 0.915–0.920 |
| Refractive index at 40 ℃ | 1.4480–1.4492 |
| Insoluble impurities per cent by mass | Max 0.05 |
| Saponification value | Min 250–260 |
| Iodine value | 4.1–11 |
| Unsaponifiable matter % by mass, max | 0.2–0.5 |
| Specific gravity at 30 ℃ | 0.915–0.920 |
| Polenske Value | Min 13 |
| Total Plate Count | < 0.5 |
| Color | Water clean |
| Odor and Taste | natural fresh coconut scent, free of sediment, free from rancid odor and taste |

**Source:** APCC (2003)

Due to its better natural sense and nutritional factor compared to the RBD treated oil, VCO has gained wide attraction among the public and scientific

community as functional food oil. Some different physicochemical properties of VCO and RBD treated CO are shown in **Table 4**.

**Table 4** Physicochemical properties of VCO and CO with RBD treat

| Properties | VCO | CO with RBD treat |
|---|---|---|
| Melting point (°C) | 24 | 24 |
| Moisture (%) | < 0.1 | < 0.1 |
| Iodine value (mg $I_2$/g) | 12–15 | 10–12 |
| Peroxide value (meq. $O_2$/kg) | 0–1 | 0–1 |
| Saponification value (mg KOH/g) | 245–255 | 250–255 |
| Phospholipids (%) | 0.1 | 0 |
| Unsaponifiable matter (%) | - | 0.19 |
| Tocopherols (mg/kg) | 150–200 | 4–100 |
| Total phenolics (mg/kg) | 640 | 20 |
| Saturated fatty acids (% TFAs) | 92 | 92 |
| Monounsaturated fatty acids (%TFAs) | 6 | 6 |
| Polyunsaturated fatty acids (%TFAs) | 2 | 2 |

Source: Gopala *et al.* (2007)

### 1.2.4 Coconut milk

To produce VCO, firstly coconut milk has to be separated from fresh coconut meat by wet extraction method, and coconut milk emulsion is then further destabilized to separate oil. Wet extraction is conducted by extracting the endosperm of mature coconuts using some instruments such as screw press or hydraulic press (Hagenmaier *et al.,* 1983; Seow and Gwee, 1997). Chemical composition of fresh coconut milk is varied depending on range of geographical sources, maturity of coconut fruits and methods of extraction (Grimwood, 1975). Gonzalez and Tanchuco

(1977) reported the specific gravity (1.0029–1.0080), surface tension (97.76–125.43 dyne cm$^{-2}$), viscosity (1.61–2.02 mPas), refractive index (1.3412–1.3446), and pH (5.95-6.30) of coconut milk. The carbohydrates mainly present in coconut milk are sugars, primarily sucrose, and some starches. Freshly extracted coconut milk contains small amounts of water-soluble vitamins, involving vitamin B and ascorbic acid (Seow and Gwee, 1997).

Coconut milk is a natural O/W emulsion in which coconut oil droplets are dispersed throughout an aqueous continuous phase and stabilized by coconut proteins and phospholipids. The protein content in coconut milk is *ca.* 5–10 % (on dry basis), in which 70 % of these proteins, most are albumins and globulins, are located at the oil-water interfacial areas and 30 % of the proteins are in the aqueous phase (Hagenmier *et al.,* 1972; Samson *et al.,* 1971). Coconut milk is thermodynamically unstable and readily separates into cream and serum layers known as coconut cream and coconut skim milk, respectively (Hagenmaier *et al.,* 1980). The physical instability of coconut milk is suspected to be a consequence of inadequate quantity and quality of coconut proteins. Coconut proteins are highly sensitive to temperature, and denature at the temperature above 80 ℃ (Gonzalez, 1990; Kwon *et al.,* 1996). Coconut milk showed several endothermic transitions over the temperature range of 80 ℃ to 120 ℃, suggesting to a complex protein composition and thermal denaturation behavior of the coconut proteins (Kwon *et al.,* 1996; Seow and Goh, 1997). Heat denaturation of the coconut protein was accelerated at the acidic and basic pH regions (Onsaard *et al.,* 2005), whereas better heat tolerance could be observed in s presence of sugars, polyols, and salts (Seow and Goh, 1997).

### 1.2.5 Extraction of VCO

To produce VCO, coconut milk emulsion has to be broken down. Destabilization of emulsion proceeds through three steps, including creaming, flocculation, and coalescence. Creaming is a separation of two phases, which are a lower specific gravity phase of oil at the top and a higher specific gravity phase of serum at the bottom. Flocculation is a clustering of oil droplets without rupturing of the interfacial films. Coalescence is the most severe destabilization, in which the interfacial films are ruptured to let oil drops to be merged together, resulting in a

released oil phase (Onsaard *et al.,* 2005). To destabilize the coconut milk emulsion, various techniques are implemented including:

**1.2.5.1 Fermentation:** Fermentation is a traditional method typically conducted by mixing coconut milk with boiled water and incubating at room temperature for 24–48 h (Tripetchkul *et al.,* 2010; Satheesh and Prasad, 2014). Upon fermentation, oil releasing from the coconut milk emulsion matrix could be accomplished by gravitational force (Raghavendra and Raghavarao, 2010) and activity of airborne lactic acid bacteria (Srivastava and Semwell, 2013). Lactic acid bacteria used lactose present in coconut milk and produced lactic acid, leading to alter acidity of the system to around pH 4, that coconut proteins were easily coagulated (Tangsuphoom and Coupland, 2008). It has been suggested that the coconut milk emulsion was destabilized, when pH was adjusted to the range of 3–5.6 (Marina *et al.*, 2009a).

Che Man *et al.* (1997) could extract VCO with the yield of 95 % by using a pure culture of *Lactobacillus plantarum* 1041 IAM. In this study, grated coconut meat were mixed water (30, 50 and 70 ºC) at the ratio of 1:1, 1:2 and 1:3, before allowing to settle for 2 to 6 h. The most efficient coconut cream separation was observed at the mixing ratio of 1:1, water temperature of 70 ºC, and incubation time of 6 h. Oil separating was supposed due to ability of *L. plantarum* to ferment sugar and produce considerable amount of lactic acid that effectively destabilized emulsion, thereby liberating the oil.

Microbes isolated from fermented coconut milk could be employed to produce VCO. The commercial yeast used for making VCO could break down the carbohydrate and sugar but not the protein and fat. Destroying the linkages between protein, fat and carbohydrate could be achieved by inoculation microbes having proteolysis and amyloidal enzymes to coconut milk (Meroth *et al.,* 2003). Centrifugation is always further applied to separate VCO.

Fermentation is a low cost technique, but requires long time to separate VCO. With a prolonged fermentation time, wild microorganisms might grow and thereby producing several compounds which influenced to quality of the oil. The unprotected freshly coconut milk under the unhygienic environmental condition

resulted in a low quality of VCO with high free fatty acid and peroxide contents (Meroth *et al.,* 2003). From fermentation means, VCO possessed sour smell and rancidity taste that might lead to unacceptability of consumer (Tripetchkul *et al.,* 2010). By this means, moreover, rather low oil recovering efficiency was given.

**1.2.5.2 Thermal cycling technique:** To separate oil through thermal cycling means, coconut milk is exposed to low temperature condition, *ca.* 10 ℃ and -4 ℃ for chilling and freezing process, respectively. Cooling affects to crystallize a part of fat phase, and fat crystals then may penetrate into other oil droplets, resulting in emulsion destabilization through partial coalescence. When oil and water phases are more crystallized in freezing step, emulsion destabilization becomes more sever, and then oil separation is completed after thawing (Cancel, 1979). In some cases, centrifugation may be implemented before chilling and thawing to allow better packing of the coconut oil globules to enhance oil recovery efficiency (Walstra, 2003). Robledano-Luzuriage and Krauss-Maffei have established a process for VCO extraction through freeze-thaw technique. In this process, fresh coconut kernel is comminuted and pressed to obtain coconut milk which is then centrifuged to obtain cream, skim milk and some solid residues. The cream is further subjected to enzymatic action under a closely controlled temperature and pH condition. Finally, the cream is centrifuged again to obtain the oil (Marina, 2009a).

**1.2.5.3 Enzymatic extraction:** Enzymatic assisted extraction is an emerging technology used to recover oil from many plant materials such as avocado, coconut, corn germ, rapeseed, soybean, and sunflower. Enzymatic aided means could provide high recovery yield (*ca.* 80 %) with a good quality of the oil (McGlone *et al*., 1986; Che Man *et al.,* 1996). Enzymatic aided extraction can be operated more flexible and safer than conventional extraction, in term of absence of flammable solvents, less operation costs, and offering a possibility to process with different crops. Mild condition of enzyme aided extraction could produce oils with high quality that needed no further refining process (Lanzani *et al.,* 1991). Enzymes can facilitate cell wall degradation, thereby promoting oil extraction efficiency. The use of several enzymes, such as cellulases, hemicellulases, pectinases, amylases, and proteases to prepare VCO has been reported (Lanzani *et al.,* 1991). Some researchers have

reported synergistic effect of multiple enzymes to recover oil from plant materials (Dusterhoft *et al.,* 1993).

Cell wall degrading enzymes can be used to extract oil by solubilizing the structural cell wall components of oil seeds. This concept has already been commercialized for a production of olive oil, and has also been investigated for some oil bearing materials. Regarding coconut, some studies have been conducted by using several enzymes as summarize in **Table 5**. Carbohydrase could enhance a degradation of structural components, *i.e.* mannan, galactomannan, arabinoxylogactan, and cellulose, resulting in improve oil recovery yield. Proteolytic enzymes could facilitate hydrolyzing of structural fibrous protein in which fat globules were embedded, thereby improving oil liberating process.

**Table 5** Enzyme aided extraction on oil recovery yield of CO

| Enzyme | Concentration or activity | Oil yield (%) |
|---|---|---|
| Control (aqueous without enzyme) | | 12.0 |
| Pectinase (Clarex) + α-amylase (Tanase) + protease (HT-proteolytic) | 0.1:0.1:0.1 % | 80.0 |
| Pectinase (Irgazyme) + α-amylase (Tanase) + protease (HT-proteolytic) | 0.1:0.1:0.1 % | 89.3 |
| Pectinase (Petcimex) + pectinase (Clarex) + alpha-amylase (Tanase) + protease (HT-proteolyic) | 0.1:0.1:0.1:0.1 % | 87.6 |
| Pectinase (Clearzyme) + pectinase (Clarex) + α-amylase (Tanase) + protease (HT-proteolytic) | 0.1:0.1:0.1:0.1 % | 89.4 |
| Pectinase (Rohapec) + α-amylase (Tanase) + protease (HT-proteolytic) | 0.1:0.1:0.1 % | 83.5 |
| β-glucanase (brew-n-zyme) | 0.3 % | 14.4 |
| β-Glucanase (brew-n-zyme) + pectinase (Clarex) +α-amylase (Tanase) + protease (HT-proteolyic) | 0.1:0.1:0.1:0.1 % | 93.8 |

**Source:** Barrios (1990)

**1.2.5.3.1 Proteases:** Proteases can enhance hydrolysis of peptide linkages between amino acids in polypeptide chain of protein molecules (Hou *et al.,* 2004). Proteases belong to the class of hydrolases that catalyze hydrolysis reaction of various bonds with a participation of water molecule. Proteases can be used in organic synthesis to resolve a pair of enantiomeric forms in racemic mixtures through kinetic resolution where one enantiomer in the mixture is more rapidly transformed than the other. Protease catalysts can resolve enantiomers through a variety of reactions such as hydrolysis of esters or amides of carboxylic acid, esterification or transesterification reactions and amide/peptide bond formation (Nagayasu *et al.,* 1994).
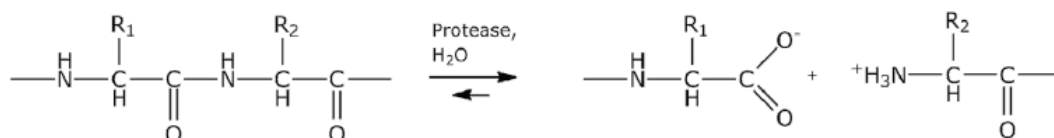


**Figure 1** Proteolysis of a peptide bond

Source: Hou *et al.* (2004)

Alcalase, the protease produce by *Bacillus licheniformis*, is commercially used prominently in detergents since the 1960s (Liceaga-Gesualdo and Li-Chan 1999). Main component of Alcalase is subtilisin A (generic name, Subtilisin Carlsberg), which is an endopeptidase. It can be employed in food preparation as approved by the FAO/WHO, JECFA and FCC (Novo Nordisk 1995). Alcalase can provide extensive proteolysis with less bitterness of protein hydrolysates compared with other proteases, making its popularity to be used in food processing (Liceaga-Gesualdo and Li-Chan 1999). Alcalase possesses a broad specificity and cleaves many types of peptide bonds, preferentially those with a hydrophobic side chain on the carbonyl side. It has a broad pH activity profile with a maximum at pH 8–9 for typical food protein substrates. It is quite thermostable and can be used at temperatures up to 70 ℃. Alcalase is typically used at an application temperature of 55–60 ℃. To inactivate Alcalase activity, increasing temperature is widely conducted in industrial practice, since it allows an almost instantaneous arresting of the proteolytic reaction in a batch hydrolysis process (Hou *et al.,* 2004).

The coconut milk emulsion was partially stabilized by coconut proteins (Seow and Gwee, 1997; Tangsuphoom and Coupland, 2008). Protease could enhance demulsification of coconut milk by hydrolyzing interior peptide bonds of the protein residues, resulting in shorten protein/peptides structures with inferior emulsifying property (Meroth *et al.,* 2003). The fractured proteins/peptide chains tended to move towards aqueous phase, thereby facilitating oil liberation from the coconut milk emulsion matrix (Rosenthal *et al.,* 1996). Proteolytic enzymes could enhance disruption of cytoplasmic network by degrading protein molecules covering around oil bodies, thereby promoting oil separation from plant cells (Rosenthal *et al.,* 1996; Jiang *et al.,* 2010). Aspartic protease, an endoprotease with a potent ability to degrade interfacial films of coconut proteins, could promote VCO recovery effectively (Raghavendra and Raghavarao, 2010). Yoon *et al.* (1991) reported an improvement in oil liberation from soybean using only proteolytic enzymes which resulted in a final yield of 86 % compared to 62 % in the process carried out without enzymes. In the case of rapeseed, an extraction oil yield of 78 % was obtained by using protease (Lanzani *et al.,* 1975). Extraction temperature had a crucial role in enzyme aided extraction process. The optimum temperature for aspartic protease was 37 ℃. Using protease in combination with other enzymes, *i.e.*, polygalacturonase and α-amylase, could rise coconut oil recovery yield up to 80 % (McGlone *et al.*, 1986; Barrios *et al.*, 1990).

### 1.2.6 Coconut fruit maturity

Physicochemical characteristics of the extracted oils have important role on their stability and utilization. Intrinsic factors, *e.g.,* plant cultivars and maturity (Umar *et al.,* 1996; Gucci *et al.,* 2004; Baccouri *et al.,* 2008) and extrinsic factors, *e.g*., extraction method (Seneviratne and Dissanayake, 2009; Marina *et al.,* 2009b; Raghavendra and Raghavarao, 2010) crucially affected the properties of oils. Different coconut cultivars had their own unique characteristics including fatty acid composition attributed to several factors such as location, age of fruits (Laurels *et al.,* 2000), and harvested time (Balleza and Sierra, 1976). Different fatty acid compositions were found for olive oils recovered from fruits with dissimilar cultivars (Temime *et al.*, 2006), which was expected due to different genetic factors and

environmental conditions during fruit ripening process (Fedeli, 1977; Lavee and Wodner, 1995).

Coconut fruit takes between 11 and 12 months to reach full maturity. At 5 months, coconut kernel begins to form a thin layer of jelly around inside of endocarp, and a shell encloses tender, clear, and sweet water. At this time the water is under pressure. During a ripening process, the pressure is released and the water is partially replaced by the kernel (**Figure 2**) (Jayalekshmy *et al.,* 1986). At 10 months old, the endosperm growth is *ca.* 77.7 % with a lipid content of *ca.* 20.22 %, and after 12 months old the endosperm is fully growth with a lipid content of *ca.* 32.64 % (Jayalekshmy *et al.*, 1986). At a full maturity stage (12 months), coconut water represents between 15−30 % of the weight of nut. The most significant change during the ripening process is a volume of the coconut water (Chikkasubbanna *et al.*, 1990; Jackson *et al.*, 2004). With increase maturity, there is an increase in nut water holding capacity until the kernel begins to form a jelly inside the cavity of fruit. Then, the water volume decrease as it is gradually used by the fruit to form kernel. As maturity approaches, the fibrous mesocarp begins to dry and reddish brown.
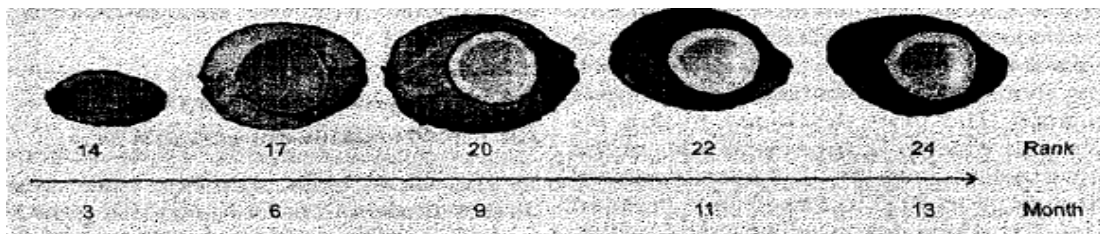


**Figure 2** Growing of coconut fruit
Source: Jayalekshmy *et al.* (1986)

Jackson *et al.* (2004) reported increased fat content of coconut fruit with increased fruit maturity. Accumulation of protein and ash with increasing coconut maturity was also observed. Increasing of protein content might be associated with a development of endosperm from a watery jelly like to a harder white meat like (Purseglove, 1972). With different maturity stages, chemical compositions of coconut fruits are affected (see **Table 6**).

**Table 6** Proximate compositions and dietary fiber content of coconuts at different maturities

| Compositions (% dry matter basis) | 10 months old | 12 months old | More than 12 months old |
|---|---|---|---|
| Ash (% DM) | 7.94 | 1.15 | 2.11 |
| Crude protein[a] (% DM) | 9.36 | 7.10 | 4.93 |
| Total lipid (% DM) | 20.22 | 32.64 | 30.71 |
| Lignin (% DM) | 0.97 | 6.69 | 3.50 |
| Hemicellulose (% DM) | 3.09 | 6.73 | 17.38 |
| Cellulose (% DM) | 8.09 | 7.09 | 6.51 |

Source: modified from Santoso *et al.* (1996)

[a] $6.25 \times \%$ N

Maturity of plants could affect fatty acid composition of the extracted oils. For sunflower seed oil, the content of C18:1 was 12 % TFAs at 7 days after the initiation of flowering (DAF), increased to 59.6 % TFAs at 14 DAF, and then diminished to 31.4 % TFAs by 56 DAF, whereas the C18:2 was present 48 % TFAs at 7 DAF, decreased to 23 % TFAs by 14 DAF, and turned to increase to 59.2 % TFAs by 56 DAF (Robertson *et al.,* 1978). Considering on extra virgin olive oil, the C18:1 content decreased gradually, whereas the C18:2 level increased as increase fruit ripened stage (Baccouri *et al.,* 2008). The diminishing in C16:0 content of extra virgin olive oil during ripening process was reported by Baccouri *et al.* (2008), supposed since a dilution effect (Gutiérrez *et al.,* 1999).

### 1.2.7 Soybean oil (SBO)

SBO is one of the major cooking oils used in household and industrial levels. Fatty acid compositions of SBO are illustrated in **Table 7**. Due to its high C18:2 content, SBO is unstable to lipid oxidation and high temperature condition of frying process. To reduce contents of polyunsatureted fatty acids (PUFAs) of SBO, partial hydrogenation, addition of antioxidants (Warner *et al.,* 1985; Frankel *et al.,*1980;

Snyder *et al.,* 1986), mutation breeding (Haumann, 1990; Scowcroft, 1990), and chemical modification through interesterification technique (Neff *et al.,* 1992, 1993) have been implemented to improve SBO stability. However, partial hydrogenation has less appealing process, because it can cause transisomerization of fatty acids with adverse effect to human health (Mensink and Katan, 1990; Zock and Katan, 1992). Mutation breeding of plants is still questionable and does not appear in the market, whereas interesterification of SBO with other stable vegetable oils consumes high processing cost.

**Table 7** Fatty acid compositions of SBO

| Fatty acids | Content (% TFAs) |
| --- | --- |
| Lauric (C12:0) | 0–0.1 |
| Myristic (C14:0) | 0–0.2 |
| Palmitic (C16:0) | 9.7–13.3 |
| Palmitoleic (C16:1) | 0–0.2 |
| Stearic (C18:0) | 3.0–5.4 |
| Oleic (C18:1) | 17.7–28.5 |
| Linoleic (C18:2) | 49.8–57.1 |
| Linolenic (C18:3) | 5.5–9.5 |
| Arachidic (C20:0) | 0.1–0.6 |
| Gadoleic/ Gondoleic (C20:1) | 0–0.3 |
| Eicosadienoic (C20:2) | 0-0.1 |
| Behenic (C22:0) | 0.3-0.7 |
| Docosenoic (C22:1) | 0-0.3 |
| Lignoceric (C24:0) | 0–0.4 |

Source: Firestone (1990)

### 1.2.8 Emulsion

Emulsion is defined as a system consisting of two immiscible liquids (usually oil and water), with one of the liquids dispersed as small spherical droplets in the other. A considerable number of natural and processed foods consist either partly or wholly as emulsions, or have been in an emulsified state sometime during their production, *e.g*., milk, cream, beverages, infant formula, and so on (Waraho *et al.,* 2011).

#### 1.2.8.1 Physical stability of emulsion

The term of emulsion stability refers to ability of emulsion to keep its properties unchanged over a certain period of time. Emulsion is a thermodynamically unstable system given kinetic stability by the material adsorbed at the interface. The mechanisms by which an emulsified oil can return to thermodynamic stability (two macro-phases) include creaming, flocculation, coalescence, and, frequently less significantly, disproportionation. Creaming is induced by a different density between dispersed and continuous phases, and leads to a bulk separation under gravity. Flocculation is a process that dispersed droplets aggregate to form a cluster but each droplet remains their integrity. Coalescence is similar to flocculation in that it requires droplet-droplet contact, but in this case individual droplets are merged together and the Laplace pressure forces the droplets to rapidly take on a spherical shape. It is important to note that whilst these mechanisms can be identified and discussed in isolation, in a real food system they are highly coupled, for example, because of the larger effective droplet sizes formed during flocculation and coalescence, creaming is often enhanced (Demetriades *et al.,* 1997).   Concentrations and types of oils employed as a dispersed phase could affect emulsion dispersibility. **Table 8** shows time dependence on particle size of emulsions made from different kinds of oil.

**Table 8** Effect of different oil phases on particle size of the emulsions stored at 22 °C for various times

| Oil | Mean particle size (nm) | | | |
|---|---|---|---|---|
| | 0 Month | 1 Month | 2 Month | 3 Month |
| **Almond oil** | 143±3.3 | 144±2.8 | 143±2.3 | 145±2.7 |
| **Sesame oil** | 142±3.1 | 142±2.3 | 140±2.8 | 141±3.1 |
| **Castor oil** | 160±4.5 | 163±4.2 | 161±3.8 | Unstable |

Source: Benita and Levy (1989)

Driscoll *et al*. (2001) demonstrated that MCFAs showed a great miscibility and might provide the emulsion with more stable compared to the counterparts containing a single long-chain triacylglycerides. Granger *et al.* (2006) observed different interface characteristic and rheological property of O/W emulsions, when dissimilar types of oils (hydrogenated oleic oil, refined coconut oil and refined palm oil) were employed. Better stability of the emulsions produced from less unsaturated oils compared to the counterparts made from PUFAs traditional oils, such as soybean and sunflower oils was evident, which was attributed to emulsifying activity of MCFAs that effected to enhance physical stability of the system (Granger *et al.*, 2006). Substitution of SBO with palm kernel olein up to 30 % provided the emulsions with improved stability along 30 days, which was attributed to a structural rearrangement by a strong network formation of the droplets in emulsified matrix (Hayati *et al.,* 2007). Significant contents of short and medium chain fatty acids were believed to partly contribute to a structural rearrangement, and thus led to a better miscibility between the dispersed and continuous phases (Hayati *et al.,* 2007).

Oil content had a crucial role on emulsion dispersibility. Guo and Mu (2011) observed inferior emulsion stability, when oil fraction was higher than 0.35. Emulsion stability decreased with increased oil volume fraction, because of the increase in packing fraction of oil droplets (Dickinson and Golding, 1997). With increased packing fraction, collision frequency between oil droplets was increased and led to enhance drop aggregation, thereby decreasing emulsion dispersibility. Rheological property of emulsion could affect to stability of the system. High viscosity of continuous phase could limit a motion of oil droplets and decrease a

frequency collision among oil drops, leading to lowered creaming rate (Hunt and Dalgleish, 1994).

### 1.2.8.2 Oxidation stability of emulsion

Lipid oxidation is a major chemical deterioration in oil containing products, by affecting undesirable changes in flavor, texture, appearance, and nutritional quality (McClements and Decker, 2000). Emulsion is prone to lipid oxidation, regarded due to a presence of high surface area that increases interaction between lipid and aqueous soluble prooxidants (Chaiyasit *et al.,* 2007). Emulsion possesses lipid oxidative mechanism differently from bulk lipid, because emulsion is multiphasic system consisting of aqueous phase which contains both prooxidants and antioxidants and oil-water interface that impact interactions between oil and water components (McClements and Decker, 2000; McClements, 2005). To retard lipid oxidation in emulsions, numerous techniques have been studied including antioxidant agent adding and interfacial engineering to control composition, thickness or charge of the interfacial layer (McClements, 2005).

### 1.2.9 Salad dressings

Salad dressing is a widely consumed oil containing product. It is present in a form of O/W emulsion with a high content of oil (*ca.* up to 30 %) and consists with other condiments such as sugar, salt, and lemon. Wheat and dairy products (*e.g.,* milk and sweet condensed milk), fruits, vegetables, eggs, herbs and spices (*e.g.,* pepper and garlic) may be also added (Thai Community standards, 2004). Salad dressing is always produced in two different finished forms, *i.e.,* emulsified and separated forms. Emulsified or one phase pourable dressing is prepared by homogenizing or blending to obtain a creamy, non-separating consistency product. Homogenization is responsible to reduce oil droplet size to provide a smooth and creamy texture. Separating salad dressing, on the other hands, has a separate oil layer above an aqueous phase. This product must be shaken before use and shows quick phase separation after pouring. Rheology plays important role on stability and acceptability in dressing products.

### 1.2.9.1 Physical stability of salad dressing

Salad dressing is O/W emulsion with high oil content, so phase separation is a significantly instability process of the product (Dalgleish, 1997; Raymundo *et al.*, 1998). O/W emulsion is a dispersed system consisting of oil droplets dispersed in an immiscible aqueous medium (McClements, 2004). Emulsion tends to break down during storage due to their thermodynamic instability involving flocculation and coalescence (McClements, 2004). Occurrence of flocculation and coalescence in an emulsion results in a modification of rheological properties due to alteration of the effective hydrodynamic volume of dispersed phase. Increase in viscosity could retard phase separation in dressing product, but a thickened mouth–feel might detrimental to sensory quality depending on the nature of the product and consumer preference (Mihov *et al.,* 2012).

Emulsifier plays important role on facilitating formation and enhancing stability of emulsion. Emulsifier should be introduced in enough content to cover total surfaces of oil droplets to ensure system stability. Traditionally, egg yolk is used as emulsifier in dressing, and other emulsifiers such as milk and soy proteins may be employed for low or non-fat products. Polysaccharides, such as xanthan gum, galactomannans, modified starches, and pectin can also be employed to stabilize dressing. Polysaccharides could control the extent of flocculation and coalescence through manipulation of rheological properties of the aqueous phase as well as to control interaction potentials between dispersed particles, leading to improve product dispersibility (Dickinson, 2003; Klinkesorn *et al*., 2004).

### 1.2.9.2 Chemical stability of salad dressing

With high oil content and presence of large interfacial areas, salad dressing is rather prone to lipid oxidation. SBO, the oil generally used to produce salad dressing, contains large amounts of PUFAs that are readily oxidized (Jung, *et. al.,* 2007). Lipid oxidation is predominantly considered to cause a quality deterioration of oils-containing food products, including appearance, taste, flavor, texture, shelf-life and nutritional profile (Min and Boff, 2002). Lipid oxidative involves the interaction between unsaturated lipids and oxygen-active species, and may be catalyzed by light, heat, enzymes, metals, metalloproteins and

microorganisms (Jung *et. al.,* 2007). Formation of hydroperoxides may break down to a variety of nonvolatile and volatile secondary products. Oxidation of emulsified lipid is mechanistically different from that occurring in bulk oil, in which organization of lipid molecules within emulsion system and their interactions with other food components influences their susceptibility to oxidative reaction (McClements and Decker, 2000). To retard oxidative deterioration in dressing, antioxidants are normally added (Halliwell *et al.,* 1995).

Polysaccharides play important roles as thickening, stabilizing and gelling agents in many foods, and they are very often used to improve stability and textural properties of emulsified product (Dickinson, 1998). Xanthan gum, an extracellular polysaccharide produced by *Xanthomonas campestris*, is effective stabilizers widely used in dressing formulations, by giving appreciate product characteristic such as creamy mouth-feel, high yield stress for suspension ability, thickness, and strong pseudoplasticity (Nor Hayati *et al.,* 2007). It has been suggested that polysaccharides could retard lipid oxidative in emulsion system (Shimada *et al.,* 1992). Antioxidative activity of polysaccharides seems to be mainly due to their ability to increase viscosity of continuous phase, leading to a reduction in oxygen diffusion rate and oil droplet collision probability (Shimada *et al.,* 1996). Polysaccharides could also exhibit chelating ability, thereby retarding oxidative reaction of the system (Shimada *et al.,* 1992, 1996).

### 1.2.10 Maltodextrins (MD)

MD is a hydrolysis product of starch consisting of α-(1,4) linked D-glucose oligomers and/or polymers. Generally MD is produced through acid, enzyme, or acid/enzyme combination hydrolysis process (Reineccius, 1991; Shahidi and Han, 1993). MD can be classified based on average molecular dextrose equivalent (DE), which is the index indicating reducing power of starch derived polysaccharide/oligosaccharides compared with D-glucose on a dry weight basis (Wang and Wang, 2000). Normally, MD has a DE value less than 20 (Reineccius, 1991; Shahidi and Han, 1993). MD with different DE values exhibits different physicochemical properties, including solubility, freezing temperature, viscosity, *etc.* (Dokic *et al.,* 2004). MD with a same DE may also has different properties depending

on the hydrolysis procedure, source of starch (maize, potato, rice), and amylose to amylopectin ratio (Dokic *et al.,* 2004). MD is widely used in food emulsions as stabilizers, as well as texture modifier to improve stability of emulsified product (Chronakis and Kasapis, 1995; Chronakis, 1997; Dokic *et al.,* 2004; Hardas *et al.,* 2000; Hogan *et al.*, 2001). MD predominantly plays role on emulsion stability by modifying viscosity or gelation of a continuous phase of the system (Dickinson, 2003). Emulsions containing MD as a stabilizer always require an additional emulsifying agent (Hogan *et al.,* 2001). For emulsion containing surfactant and polysaccharide, stability of the system depends on interaction between surfactant and polysaccharide at both interface area and aqueous phase (Dickinson, 2003). Small molecular surfactants could bind to MD by inserting their non-polar tails into a helical coil of MD chain, resulting in alteration of functionality of both the surfactant and MD in emulsion system (Wangsakan *et al.,* 2001, 2003). Molecular characteristics of MD, such as concentration and chain length, could affect rheology and stability of emulsion. Source of starch is one of crucial factors influencing the properties of MD, because starches from various botanicals have dissimilarity in chain-length distribution and molecular weight. Considering on utilization of MD on stability of dressing products, Klinkesorn *et al*. (2004) found that the dressing containing the corn MD with DE of 36, 25, 20, 15 and 10 begun to flocculate, when the MD concentrations were 35, 21, 21, 17 and 13 % (w/w), respectively.

### 1.2.10.1 Tapioca maltodextrin (TMD)

Tapioca starch is obtained from roots of cassava plant, which is found in equatorial regions between the tropics of Cancer and Capricorn (William *et al.,* 2009). Tapioca is a shrubby perennial crop which is ease of plantation and low input requirement. It can grow in all soil types, but root formation is better in loose structured soils, such as light sandy loams and/or loamy sands. It can grow even in infertile soil or acid soil. Typical mature roots (9–12 months old) have an average composition of 60–70 % water, 30–35 % carbohydrate, 1–2 % fat, 1–2 % fiber, and 1–2 % protein with trace quantities of vitamins and minerals (Balagopalan *et al*., 1988; Rojanaridpiched, 1989). Mature roots possess starch content from 15 to 33 % depending on the climate and harvest time.

Tapioca starch is differentiated from other starches by its low level of residual materials (especially for proteins and lipids), lower amylose content than for other amylose-containing starches, and high molecular weights of amylose and amylopectin (Swinkels, 1985). Typically, cassava starch contains 17–20 % amylose, whereas corn and rice consist of 0–70 % and 0–40 % amylose contents, respectively. The amylose molecules of cassava starch are not completely unbranched as indicated by lower β-amylolysis limit than those observed for corn, potato, rice and wheat starches. In addition, cassava amylose has a higher molecular weight than other starches (Balagopalan *et al.,* 1988; Rojanaridpiched, 1989). The low amylose, lipid and protein contents combining with high molecular weight of amylose make tapioca is a unique native starch for food and industrial application. The application primarily involves enzyme catalyzed hydrolysis, providing various products by varying enzyme types, degree of hydrolysis and derivatization. Important products obtained by direct hydrolysis of starch are sugar syrups, *i.e.,* glucose– and maltose syrups (Reineccius, 1991; Shahidi and Han, 1993). The syrups produced from tapioca starch are bland taste, clean flavor, high purity and ease of cooking due to a lower gelatinization temperature.

Considering on utilization of TMD in emulsion model, it has been reported that critical flocculation concentration (CFC) of TMD with DE 16, 12 and 9 were 11, 7 and 5.5% (w/w), respectively (Udomrati *et al.,* 2011). By applying the TMD with DE of 12 to 9 to the emulsions, coalescence could be inhibited when the concentrations were greater than 40 and 35 % (w/w), respectively. While, the emulsions containing the TMD with DE 16 showed creaming throughout the concentration range of 15–50 % (w/w).

## 1.3 Objectives

The present work aimed to elucidate the effects of protease aided extraction on oil recovery yield and characteristics of VCO, by comparing with conventional methods, *i.e.*, fermentation and freeze-thaw cycling techniques. Influence of coconut fruit maturity on chemical characteristics of VCO was also studied. After that, the physicochemical stability of the model O/W emulsion prepared using VCO as a dispersed phase was examined by comparing with the counterparts made from SBO.

Finally, VCO was used to produce salad dressings, and physicochemical stability and characteristics of the VCO dressings were observed in parallel with the dressings made from SBO. Furthermore, effect of TMD adding at various concentrations on the stability of dressing samples was also elucidated.

# CHAPTER 2

# EFFECT OF DIFFERENT EXTRACTION PROCESSES
# ON OIL RECOVERY EFFICIENCY AND CHARACTERISTIC OF
# VIRGIN COCONUT OIL

## 2.1 Abstract

The effect of different oil recovering techniques including conventional (*i.e.*, fermentation and thermal cycling) and protease aided extractions on oil recovery yield and properties of virgin coconut oil (VCO) was observed. The highest oil extraction yields derived by enzyme aided–, thermal cycling–, and fermentation methods were 93.5%, 79.0%, and 74.9%, respectively. Lauric acid was predominantly present in all of the VCOs with the content ranging of 49.51–51.72% of total fatty acids. Enzyme aided extraction provided the oil with greater amount of unsaturated fatty acids compared to the oils given by the conventional methods. The highest contents of health promoting microconstituents, *i.e.,* tocopherols and phenolic compounds, were found for the oil recovered through fermentation means. The majority phenolic compounds present in the VCO were catechin, gallic, vanillic, and *p*-coumaric acids. Chemical properties of all VCOs produced in this work conformed to the standard of the Asian pacific coconut community suggesting that the produced oils could be commercially available.

## 2.2 Introduction

Coconut oil is widely used in many industries including food, pharmaceuticals, and cosmetics due to its several advantages, such as richness in medium chain fatty acids (MCFAs) with a good digestibility and antiviral activity (Che Man and Marina, 2006; Marina *et al.,* 2009a). Coconut oil is traditionally recovered through dry extraction, and the derived oil has to be further treated through a refining, bleaching, and deodorizing (RBD) process. The RBD process affects to deteriorate oil qualities in several traits involving masking desirable natural odor, generating high content of free fatty acid (FFA), and destroying health promoting microconstituents (Nevin and Rajamohan, 2004; O'Brien, 2004; Villarino *et al.,* 2007). High content of FFA

affected to shorten shelf-life and impair qualities of the oils by promoting oxidative rancidity and oil degradation when used in frying process (Che Man *et al.,* 1997; Raghavendra and Raghavarao, 2010). To conquer this drawback, wet extraction conducted by separating coconut milk from fresh coconut meat and consequently breaking the milk emulsion to liberate oil phase is a promising method. The coconut oil produced from wet extraction is referred to virgin coconut oil (VCO), which is defined as the oil obtained from fresh, mature coconuts without any chemical refining (Shilhavy and Shilhavy, 2004; Villarino *et al.,* 2007). Better sensory characteristic and higher nutritive value were guaranteed for VCO than the RBD treated oil, making popularity of VCO as a functional ingredient in food processing (Shilhavy and Shilhavy, 2004; Villarino *et al.,* 2007; Marina *et al.,* 2009b).

The coconut milk extracted from the endosperm of mature coconuts is naturally present in a form of oil-in-water emulsion (O/W), which is stabilized by proteins, *i.e.,* globulins and albumins, and phospholipids (Seow and Gwee, 1997; Tangsuphoom and Coupland, 2008). To separate oil, the coconut milk emulsion has to be destabilized by breaking down the oil-water interfacial films of the lipoproteins (Sharma *et al.,* 2001). Enzymatic aided extraction is an emerging oil recovery technique offering many advantages compared to a conventional means, *i.e.* eliminate time and solvent consumption as well as provide effective recovery yield and a good quality of the derived oils (Sharma *et al.,* 2001; Marina *et al.,* 2009b; Marasabessy *et al.,* 2010; Jiang *et al.,* 2010). Enzymatic extraction is always conducted under mild temperature, so it is regarded as an eco-friendly process (Villarino *et al.,* 2007). It has been reported that enzyme aided extraction could improve oil recovery yield from various plants such as *Jatropha curcas* (Marasabessy *et al.,* 2010), rice bran (Sharma *et al.,* 2001), and peanut (Jiang *et al.,* 2010). Chemical characteristics of the extracted oils play important role on their stability and utilization. By using different extraction methods, the coconut oils possessed different fatty acid profiles, thereby affecting to their health benefit and stability (Raghavendra and Raghavarao, 2010). The present work aimed to elucidate properties of VCO recovered by different techniques, in order to produce VCO with a good recovery yield and pleasant characteristics.

## 2.3 Objective

-To optimize the condition of enzyme aided extraction on a production of VCO with high recovery yield and good qualities

-To characterize the physicochemical properties and chemical components of VCO recovered by different methods, involving conventional (fermentation and freeze-thaw cycling) and enzyme aided extractions

## 2.4 Materials and methods

### 2.4.1 Materials

Fresh coconut fruits (11–12 months old) and commercial VCO (Tropicana, Nakhon Pathom, Thailand) were purchased from a local market in Hat Yai (Songkhla, Thailand). Alcalase, the protease from *Bacillus licheniformis*, with an activity of $\geq 5U/g$ (1 U corresponds to the amount of enzyme that sets free 1 μmol Folin-positive amino acids and peptide (as tyrosine) per min at pH 7.0 and 37 °C, using casein as a substrate), 2,2-bipyridine and Hydranal®-Coulomat AG-Oven were products of Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, ethanol, toluene, chloroform, methanol and water HPLC grade were purchased from J.T. Baker (Center Valley, USA.). Potassium iodide, potassium hydroxide, cyclohexane, and sodium carbonate were purchased from Ajax FineChem (Auckland, New Zealand). Folin Ciocalteau's and iodine monochloride were products of Fluka (St. Louis, MO, USA). All the chemicals and solvents used were of analytical grade.

### 2.4.2 Preparation of VCO

Coconut milk was separated using a screw press (Fujica CM-SJ, Bangkok, Thailand). The grafted coconut meats were pressed for three times and the separated milk was mixed together. The coconut milk was destabilized by different techniques to produce VCO.

### 2.4.3 Oil extraction processes

#### 2.4.3.1 Fermentation method

The coconut milk was incubated at a controlled temperature of 30 ± 2 °C (water bath, Binder BD115, Tuttlingen, Germany) for 24, 36 and 48 h. The sample was then centrifuged (Beckman Coulter Avanti JE, California, USA) at $15,000 \times g$ for 15 min to separate coconut cream and aqueous phase. To obtain clear oil, the cream phase was further centrifuged at $15,000 \times g$ for 15 min. This procedure was modified from the method of Raghavendra and Raghavarao (2010).

#### 2.4.3.2 Thermal cycling method

The coconut milk was subjected to freeze-thaw program with various numbers of cycle according to the method described by Raghavendra and Raghavarao (2010) with some modifications. Initially, the coconut milk was freezed at -20 ℃ for 6 h, placed at room temperature for 30 min, and heated at 60 ℃ for 10 min in a water bath. This tempering program included 7 h and defined as 1 cycle. The coconut milk was treated for 1–4 cycles, before centrifuging at $15,000 \times g$ for 15 min. The separated cream phase was further centrifuged at $15,000 \times g$ for 15 min to release clear oil.

#### 2.4.3.3 Enzyme aided extraction means

Alcalase was introduced to the coconut milk at different concentrations (0.05, 0.1 and 0.3%, w/w), and oil extraction was conducted at 60 °C for various times (0, 15, 30, 60 and 120 min). The sample was then centrifuged at $15,000 \times g$ for 15 min, and the cream phase was further centrifuged at $15,000 \times g$ for 15 min to release clear oil. This procedure was modified from Raghavendra and Raghavarao (2010).

**2.4.4 Analyses the effects of different extraction processes on oil recovery efficiency and characteristics of VCO**

**2.4.4.1 Determination of oil recovery**

Oil recovery efficiency derived by different extraction methods was determined by the following equation (Mansor *et al.,* 2012):

$$\% \text{ oil recovery} = \frac{((\text{weight of extracted oil/weight of coconut milk}) \times 100)}{(\% \text{ oil in coconut milk})} \times 100$$

**2.4.4.2 Determination of peroxide value (PV)**

PV was quantified by the standard method of IUPAC (1992). The oil sample (5 g) was thoroughly mixed with a mixture of acetic acid:chloroform (3:2 v/v, 25 ml) and saturated KI solution (1 ml), before incubating in the dark for 1 h. After adding water (75 ml), the mixture was titrated with a standard solution of sodium thiosulfate (0.01 N) using a starch solution as an indicator.

**2.4.4.3 Determination of free fatty acid (FFA)**

FFA was determined by a titration method (IUPAC, 1992). The oil (8 g) was mixed with ethanol (50 ml), before neutralizing with NaOH (0.01 N) using phenolphthalein as an indicator. FFA of the VCO was expressed as a percentage of lauric acid.

**2.4.4.4 Determination of saponification value (SV)**

SV measurement was carried out according to the method of IUPAC (1992). The oil (2 g) was mixed with KOH solution in ethanol (0.5 N, 25 ml), before distilling for 1 h. After cooling to room temperature, the mixture was titrated with HCl (0.5 N) using phenolphthalein as an indicator.

**2.4.4.5 Determination of iodine value (IV)**

IV was determined by a standard method of IUPAC (1992). The oil (1 g) was thoroughly mixed with cyclohexane (15 ml) and Wijs solution consisting of iodine monochloride (0.5 % v/v, 25 ml), before incubating in the dark for 1 h. After

adding with KI solution (10 % w/v, 20 ml) and water (150 ml), the mixture was titrated with a standard solution of sodium thiosulfate (0.1 N) using a starch solution as an indicator.

### 2.4.4.6 Determination of total phenolic content (TPC)

TPC present in the oil sample was determined by Folin-Ciocalteu assay followed the method of Arslan *et al.* (2013) with a slight modification. Briefly, the oil (5 g) was mixed with a mixture of ethanol:water (80:20 v/v, 3 ml), before centrifuging at $5000 \times g$ for 5 min. This extraction was carried out for three times and all ethanolic extracts were combined, before evaporating until dryness using a rotary evaporator (Eyela N-1000, Tokyo, Japan). The dry matter was redispersed using a mixture of methanol:water (10:90 v/v, 1 ml), before adding with water (8.2 ml) and Folin-Ciocalteau reagent (0.5 ml). The mixture was allowed to stand at room temperature for 5 min, added with sodium carbonate solution (10 % w/v, 1 ml), and incubated at room temperature for 60 min. The absorbance at 765 nm was then read (UV-Vis Spectrophotometer, UV-1700, Shimadzu, Kyoto, Japan). TPC was calculated using a standard curve of gallic acid (0–100 μg/100 ml) and expressed as milligrams gallic acid equivalents (GAE) per kilogram of oil.

### 2.4.4.7 Determination of phenolic composition

Firstly, phenolics were extracted from the VCO followed the method described Arslan *et al.* (2013). Briefly, the oil (5 g) was mixed with a mixture of ethanol:water (80:20 v/v, 3 ml), before centrifuging at 5000×*g* for 5 min. This extraction was carried out for three times and all ethanolic extracts were combined, before evaporating until dryness using a rotary evaporator. The dry matter was redispersed using a mixture of methanol:water (10:90 v/v, 1 ml). Phenolic compounds present in the oil were identified by HPLC (Agilent Technologies 1200 series G1329A, Waldbronn, Germany), the column was a Hypersil ODS (particle size 5 μm, length 250 mm × width 4 mm) (Thermo Electron Corporation, Waldbronn, Germany), followed the method of Arslan *et al.* (2013). The flow rate was 0.85 ml/min, and the injection volume 20 μL. The total run time was 35 min. The eluents were 2 % aqueous acetic acid solution (A) and methanol (B). The gradient time program was set

as follow: 0 min 5 % B, 3 min 15 % B, 13 min 20 % B, 25 min 25 % B, and 32 min 30 % B. The absorbance at 240, 280, and 320 nm was read. Identification of each phenolic was determined based on a combination of retention time, using catechin, gallic, *trans*-ferulic, vanillic, *p*-coumaric, and syringic acids as standards.

### 2.4.4.8 Determination of total tocopherols content

Tocopherols content present in the oils was measured according to the method described by Wong *et al.* (1998). The oil (1 g) was weighed accurately into 10 ml volumetric flask. Toluene (5 ml), 2,2-bipyridine (0.07 % w/v in 95 % ethanol, 3.5 ml) and $FeCl_2$ solution (0.2 % w/v in 95 % ethanol, 0.5 ml) were added to the sample. The solution was then made up to 10 ml using ethanol and allowed to stand at room temperature for 1 min, before reading the absorbance at 520 nm. Blank was prepared in a same manner, but oil was omitted. Tocopherol content was determined using a standard curve of α-tocopherol (0–250 µg/kg).

### 2.4.4.9 Determination of fatty acid composition

Fatty acid composition was examined according to the method of Chowdhury *et al.* (2007). The oil (50 µl) was added with KOH solution (0.5 N in methanol, 1 ml) and digested by stirring in a boiling water bath for 20 min. After cooling to room temperature, the sample was added with a mixture of HCl:methanol (4:1 v/v, 0.4 ml), deionized water (2 ml) and petroleum ether (3 ml). The distinct upper layer of methyl ester was then separated carefully and dried by nitrogen gas. The sample was redispersed using chloroform (1 ml), before introducing to GC (Agilent technologies 7890A, Wilmington, USA) equipped with a flame ionization detector. Varian's capillary column (VF-5 ms, length 30 m × width 0.25 mm × particle size 0.25 µm; EZ-GRIP$^{TM}$, Wilmington USA) was used. The column was conditioned at 180 °C for 2 h to attain thermal stability before use. The temperature condition was operated as following: holding at oven temperature of 150 °C for 5 min, increasing to 190 °C with a rate of 8 °C/min, increasing to 200 °C with a rate of 2 °C, and holding at 200 °C for 10 min. Injection and detection temperatures were 250 °C. Nitrogen was used as a carrier gas with a flow rate of 20 ml/min.

### 2.4.4.10 Determination of moisture content

Moisture content in the oil was determined by coulometric Karl Fischer titration method using 831 Coulometric Karl Fischer and 728 Stirrer (Metrohm® Instruments, Canada, USA). Oil sample (1 ml) was accurately added into GL45 glass thread containing reagent for the reaction and stirred for 1 min. The titration curve was recorded on a strip chart recorder (Sam, 1998).

### 2.4.5 Statistical analysis

All experiments were carried out in triplicate with different three lots of oil, and the mean values with standard deviations were present. Completely Randomized Design was used. Statistical analysis of the data was performed by analysis of variance (ANOVA) using Duncan's multiple range test (SPSS for Windows, SPSS Inc., Chicago, IL, USA) at a 95 % confidence level.

## 2.5 Results and discussion

### 2.5.1 Effect of extraction method on oil recovery yield

Oil recovery yields provided by conventional methods of fermentation and thermal cycling techniques were shown in **Figure 3**. By using fermentation means, the extraction yield could be improved to 74.9 % by prolonging fermentation time up to 36 h (P<0.05), whereas extended incubation time for 48 h had no further improvement effect on oil recovery yield (P>0.05). Satheesh and Prasad (2014) reported that coconut oil could be released after fermentation time of 24–48 h. Upon fermentation, oil releasing from the coconut milk emulsion matrix could be accomplished by gravitational force (Raghavendra and Raghavarao, 2010) and activity of airborne lactic acid bacteria (Srivastava and Semwell, 2013). Lactic acid bacteria used lactose present in the coconut milk and produced lactic acid, leading to alter acidity of the system to around pH 4 that coconut proteins were easily coagulated (Tangsuphoom and Coupland, 2008). It has been suggested that coconut milk emulsion was destabilized by adjusting pH of the coconut milk emulsion to the range of 3–5.6 (Marina *et al.*, 2009a).
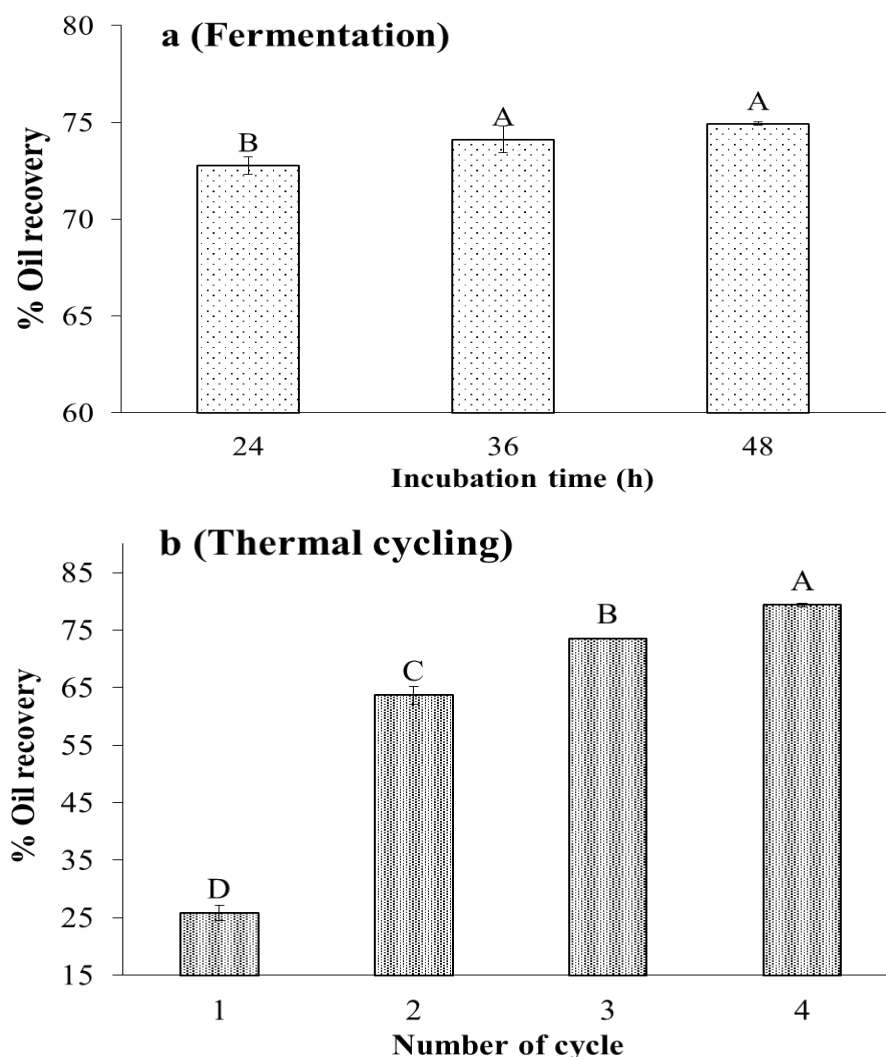
**Figure 3** Oil recovery yield provided by **(a)** fermentation technique at different incubation times and **(b)** thermal cycling technique at various numbers of thermal cycle.

Means with standard deviations ($n$=3) were shown. In each subfigure, different letters indicate significant difference between means (P<0.05).

Oil recovery yields provided by thermal cycling means were illustrated in **Figure 3b**. Increase a number of freeze-thaw cycle could enhance oil liberation, and the highest yield of 79.0 % was found after treating the coconut milk for 4 cycles (28 h of total incubation time) (P<0.05). As a result of temperature lowering, oil drops in coconut milk were solidified. Further thawing affected to deform a spherical shape of oil droplets, thereby promoting coalescence of dispersed oil droplets and leading to a releasing of oil phase from the emulsified matrix (Marina *et al.,* 2009b; Raghavendra

and Raghavarao, 2010). Reduce temperature in a chilling step could improve oil recovery yield: The extraction yields of *ca*. 65, 74, and 92 % were found for VCO production, after chilling the coconut milk at 20, 15, and 5 °C for 6 h, respectively (Raghavendra and Raghavarao, 2010).

Oil recovery efficiency derived by the enzyme aided extraction was observed at various enzyme concentrations and incubation times, and the result was illustrated in **Figure 4**. Increase enzyme concentration affected to improve oil recovery yield, especially when incubation time was increased. The highest yield of 93.5 % could be obtained by using the protease at the level of 0.3 % and incubation time of 120 min (P<0.05). Further increasing enzyme concentration and incubation time had no effect on oil recovery efficiency (P>0.05). By using the protease at the level of 0.5 % and incubation time of 180 min provide yield about 94 %. The coconut milk emulsion was partially stabilized by the coconut proteins (Seow and Gwee, 1997; Tangsuphoom and Coupland, 2008), so protease could enhance demulsification by hydrolyzing interior peptide bonds of the protein residues, resulting in shorten protein/peptides structures with inferior emulsifying property (Meroth *et al.,* 2003). The fractured proteins/peptide chains tended to move towards aqueous phase, thereby facilitating oil liberation from the coconut milk emulsion (Rosenthal *et al.,* 1996). Moreover, proteolytic enzymes could enhance disruption of cytoplasmic network by degrading protein molecules covering around oil bodies, thereby promoting oil separation from plant cells (Rosenthal *et al.,* 1996; Jiang *et al.,* 2010). Yoon *et al.* (1991) reported that using proteolytic enzymes could improve yield for soybean oil extraction, which resulted in a final yield of 86 % compared to 62 % in the process carried out without enzymes. In the case of rapeseed, an extraction oil yield of 78 % was obtained using protease-aided extraction (Lanzani *et al.,* 1975).
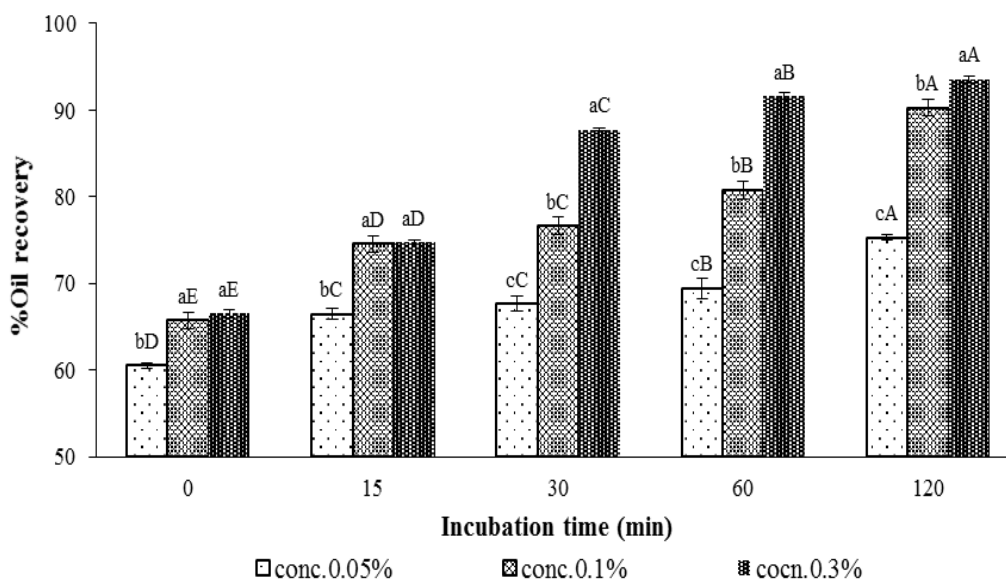
**Figure 4** Oil recovery yield provided by protease aided extraction at different enzyme
concentrations (0.05, 0.1, and 0.3 %, w/w) and incubation times.

Means with standard deviations (*n*=3) were shown. Different small (capital) letters in the same
incubation time (protease concentration) indicate significant difference between means (P<0.05).

Comparing to the traditional methods, *i.e.,* fermentation and thermal cycling
techniques, the protease aided extraction could provide higher oil recovery yield.
From the present result, the highest oil extraction yields derived by fermentation
(36 h), thermal cycling (4 cycles of total time of 28 h), and enzyme aided (0.3 %
enzyme concentration and extraction time of 180 min) methods were 74.9 %, 79.0 %,
and 93.5 %, respectively. To more elucidate the effects of different oil recovering
methods on properties of VCO, some selected properties of the VCOs prepared by the
conditions providing the highest recovery yields in each studied technique were
further examined comparing with the commercial VCO.

### 2.5.2 Characterization of virgin coconut oil (VCO) properties recovered by different techniques

Fatty acid compositions of the VCO samples were examined (see **Table 9)**. The properties of commercial VCO and the Asian pacific coconut community standards (APCC, 2003) were also illustrated in order for comparison.

**Table 9** Fatty acid compositions (% total fatty acids, TFAs) of the VCOs produced from different methods

| Fatty acid (% TFAs) | Extraction method | | | Commercial | APCC standard* |
|---|---|---|---|---|---|
| | Enzyme aid | Fermentation | Thermal cycling | | |
| C8:0 | 5.81±0.71[b] | 6.69±0.31[a] | 5.93±0.12[ab] | 6.69±0.21[a] | 5.00–10.00 |
| C10:0 | 7.16±0.61[a] | 6.86±0.41[a] | 6.48±0.16[a] | 7.10±0.20[a] | 4.50–8.00 |
| C12:0 | 50.44±0.50[ab] | 49.51±1.21[b] | 51.72±1.14[a] | 50.10±0.94[ab] | 43.00–53.00 |
| C14:0 | 17.63±0.50[ab] | 18.28±0.48[a] | 17.80±0.26[ab] | 17.24±0.26[b] | 16.00–21.00 |
| C16:0 | 8.02±0.08[b] | 8.88±0.38[a] | 8.19±0.48[b] | 8.37±0.23[ab] | 7.50–10.00 |
| C18:0 | 2.84±0.19[a] | 2.87±0.09[a] | 2.71±0.10[ab] | 2.56±0.15[b] | 2.00–4.00 |
| C18:1 | 6.29±0.30[a] | 5.92±0.18[ab] | 5.16±0.87[b] | 5.77±0.15[ab] | 5.00–10.00 |
| C18:2 | 1.87±0.18[a] | 1.00±0.30[b] | 1.53±0.38[a] | 1.91±0.07[a] | 1.00–2.50 |
| Σ saturated fatty acids | 91.87±0.42[b] | 93.08±0.11[a] | 92.85±0.86[ab] | 92.07±0.73[b] | |
| Σunsaturated fatty acids | 8.16±0.24[a] | 6.92±0.11[ab] | 6.68±0.87[b] | 6.84±0.15[ab] | |

Mean values ± standard deviations (*n*=3) were shown.

Letters within a same row indicate significant difference between means (P<0.05).

*The Asian Pacific Coconut Community (APCC) standards were shown in order for comparison (APCC, 2003).

The major fatty acid residues in all VCO samples were medium chain fatty acids (MCFAs), *i.e.,* C12:0 and C14:0. This result was in accordance with the

previous works (Chowdhury *et al*., 2007; Raghavendra and Raghavarao, 2010). By using different extraction techniques, a slight difference in fatty acid compositions of the VCOs was evident. As comparing to the VCO produced by enzyme aided extraction, the VCO recovered by traditional techniques contained less amount of unsaturated fatty acids, *i.e.,* monounsaturated fatty acids (MUFAs) of C18:1 and polyunsaturated fatty acids (PUFAs) of C18:2 for the oils recovered by thermal cycling and fermentation techniques, respectively ($P<0.05$). These tendencies are in agreement with the reported of Marina *et al.,* (2009a). Regarding to a well-recognized health benefit of unsaturated fatty acids (Reena and Lokesh, 2007), the present result implied a better nutritive value of the VCO recovered by protease assisted process than the counterparts derived by the traditional techniques. Superior nutritional value of the VCO prepared by the aid of aspartic protease compared to the commercial VCO were suggested by the higher amounts of short chain fatty acids, *i.e.,* C8:0 and C10:0 (Raghavendra and Raghavarao, 2010). In the present work, fatty acid compositions of the VCOs recovered by different techniques and the commercial VCO were within the standard values of APCC.

Next, some selected characteristics of the VCOs produced by various techniques and the commercial VCO were examined as present in **Table 10.**

**Table 10** Characteristics of the VCOs produced from different methods

| Characteristics | Extraction method | | | Commercial | APCC Standard |
|---|---|---|---|---|---|
| | **Enzyme aid** | **Fermentation** | **Thermal cycling** | | |
| **PV** (milli Equiv. $O_2$/kg fat) | $0.53\pm0.14^b$ | $0.78\pm0.08^a$ | $0.66\pm0.03^{ab}$ | $0.72\pm0.05^a$ | Max 3 |
| **FFA** (%lauric acid) | $0.12\pm0.01^c$ | $0.15\pm0.01^b$ | $0.10\pm0.01^c$ | $0.20\pm0.02^a$ | Max 0.2 |
| **SV** (mg KOH/g fat) | $269.23\pm2.85^{ab}$ | $262.77\pm4.66^b$ | $271.72\pm3.35^a$ | $269.05\pm1.61^{ab}$ | Min 250–260 |
| **IV** (g $I_2$/100g fat) | $7.62\pm0.14^a$ | $7.26\pm0.13^b$ | $7.01\pm0.08^c$ | $7.36\pm0.04^a$ | 4.1–11 |
| **Moisture content** (%) | $0.15\pm0.001^a$ | $0.15\pm0.001^a$ | $0.15\pm0.001^a$ | $0.12\pm0.001^b$ | Max 0.1 |
| **TPC** (mg GAE/kg) | $35.02\pm0.10^c$ | $59.30\pm0.39^a$ | $29.71\pm0.01^d$ | $43.59\pm0.52^b$ | - |
| **Total tocopherols** (mg/kg) | $0.003\pm0.0006^c$ | $0.022\pm0.00^a$ | $0.003\pm0.00^c$ | $0.019\pm0.0006^b$ | - |

Mean values ± standard deviations (*n*=3) were shown.

Letters within a same row indicate significant difference between means (P<0.05).

The Asian Pacific Coconut Community (APCC) standards were shown in order for comparison (APCC, 2003).

The VCO prepared by enzyme aided means had the lowest PV and FFA contents compared to oils prepared by other techniques (P<0.05), suggesting to its better initial quality. Fermentation methods resulted in the highest FFA of the VCO compared to the enzymatic and thermal cycling methods. This might be expected due to a prolonged processing time that might effect to induce hydrolysis reaction, thereby increasing FFA (Satheesh and Prasad, 2014). However, the FFA of all VCOs produced in this work conferred to the APCC standard. Note that the VCO recovered through thermal cycling, enzymatic and fermentation methods reported by Mansor *et al*. (2012) contained the higher FFA (0.29–0.35 mg KOH/g) than the present work. The highest SV was found for the VCO extracted by thermal cycling means (P<0.05). The highest IV could be observed for the oils recovered by enzyme aided means (P<0.05), which was coincident with its higher content of unsaturated fatty acids as illustrated in **Table 9**. The moisture content of VCOs recovered through different

techniques was comparable (P>0.05) within the range of 0.15%, except for commercial VCO showing the lowest moisture content (P<0.05). Moisture content is one of the important factors determining shelf-life of the product, higher moisture content faster degree of chemical deterioration (Che Man *et al.,* 1996; Marina *et al.*, 2009a). Bioactive compounds of the VCOs derived by different techniques were evaluated measuring the amounts of TPC and total tocopherols. Higher TPC and total tocopherols content were observed for the oil prepared by fermentation technique (P<0.05), implying to a greater nutritive value of the oil. Through fermentation process, pH of the coconut milk was reduced (Li and Jiang, 2007; Baublis *et al.*, 2000). This acidic condition might affect to hydrolyze bound phenolics and tocopherols, resulting in higher TPC and tocopherol contents of the derived oil. When the phenolic composition of the VCO prepared via fermentation technique was observed (see **Figure 5**), it was found that catechin, gallic, vanillic, and *p*-coumaric acids were predominant. This was in correspondence with the report of Seneviratne *et al.* (2008). The unidentified signals in the HPLC profile were supposed to be oxidized and/or bound forms of phenolic compounds (Seneviratne *et al.,* 2009; Arslan *et al.,* 2013).
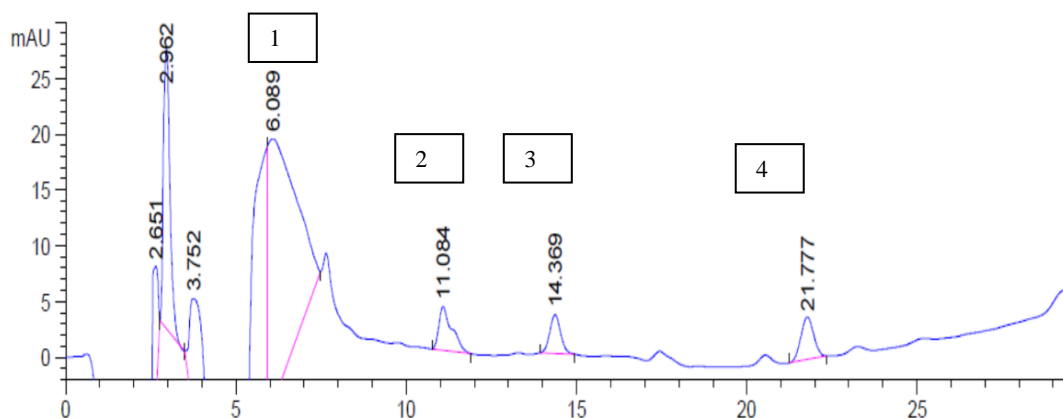


**Figure 5** HPLC chromatogram of the phenolic fraction of the VCO derived by fermentation method: (1) gallic acid, (2) catechin, (3) vanillic acid, and (4) *p*-coumaric acid.

Considering on the VCO recovered by fermentation technique, the higher amount of total tocopherols and TPC were observed. Nonetheless, a fermented off-odor could be obviously detected for the VCO recovered through fermentation means, whereas protease aided means provided the oil with pleasant natural coconut odor. It was suggested that a fermented odor affected to mask a natural desirable flavor of coconut oil and might reduce consumer acceptability (Koh and Long, 2012). Better initial quality of the lowered PV and FFA, and higher nutritive value of more abundantly present unsaturated fatty acids were found for the VCO recovered by protease aided means compared to those produced by fermentation technique. Additionally, based on economic view, the protease aided extraction could effectively enhance oil extraction yield with less production time. Therefore, extraction using protease was selected to prepare VCO in a further study.

## 2.6 Conclusion

Extraction method influenced oil recovery efficiency and chemical characteristics of the derived VCO. The highest oil recovery yield could be provided by protease aid extraction, and the obtained oil showed higher amount of unsaturated fatty acids compared to the oils extracted by fermentation and thermal cycling techniques. The VCO recovered through protease assisted means showed a greater initial quality as suggested by the lowered PV and FFA. The highest amount of total tocopherols and phenolic compounds were found for the VCO prepared by fermentation technique ($P<0.05$). The majority phenolic compounds present in the VCO were gallic, catechin, vanillic, and $p$-coumaric acids. All of the observed parameters of the VCOs were within the limits of APCC standard, suggesting that the VCOs produced in the present work could be employed commercially.

# CHAPTER 3
# EFFECTS OF FRUIT MATURITIES ON CHARACTERISTIC OF
# VIRGIN COCONUT OIL

## 3.1 Abstract

Fruit maturities had influence on chemical characteristics of virgin coconut oil (VCO). Coconut fruits at different maturities, *i.e.* 11, 12, and 13 months old referred as young–, intermediate–, and old coconut, respectively were employed to prepare VCO via protease aided extraction. The coconut at intermediate– and old age stages contained higher oil content (*ca*. 49 % w/w, wet basis) than that observed for young coconut ($P<0.05$). Lauric acid was predominantly observed in all VCOs with the content ranging from 50.44–51.81 % of total fatty acids. The VCO recovered from young fruits exhibited the best initial quality, as indicated by the lowest free fatty acid, and nutritive value, as implied by the highest contents of unsaturated fatty acids, tocopherols, and phenolic compounds.

## 3.2 Introduction

Coconut is a versatile and unique plant that can withstand any type of weather or calamities. It bears fruit all year round. Generally, coconut fruit with the age of 10 to 12 months is suitable for harvesting because of the highest oil content (Balleza and Sierra, 1976). There are many factors affecting to properties of oils including intrinsic, *e.g.,* plant cultivars and maturity (Umar *et al.,* 1996; Gucci *et al.,* 2004; Baccouri *et al.,* 2008) and extrinsic factors, *i.e.*, extraction method (Seneviratne and Dissanayake, 2009; Marina *et al.,* 2009b; Raghavendra and Raghavarao, 2010). Chemical properties of the extracted oils played important role on stability and utilization of the oils. Baccouri *et al.* (2008) reported that oxidative stability of extra virgin olive oils recovered from different olive cultivars was differed depending on the contents of indigenous tocopherol and phenolics. Fruit maturity influenced extraction yield and properties of the derived oils (Umar *et al.,* 1996; Ryan *et al.,* 2002; Gucci *et al.,* 2004; Baccouri *et al.,* 2008). By using olive fruits at different ripening degrees, fatty acid compositions of the oils were dissimilar (Baccouri *et al.,* 2008). With different maturity stages, physiology and enzyme activity in plant cells

were differed, thereby affecting to the amount and profile of several microconstituents, such as tocopherol, phenolics, squalene, and pigments, present in the extracted oils (Gutiérrez *et al.,* 1999; Ryan *et al.,* 2002; Baccouri *et al.,* 2008). Most of the researches reporting the effects of extraction method and fruit maturity on quality and characteristics of the extracted oils have been done for olive oils (Gutiérrez *et al.,* 1999; Caponio *et al.,* 2001; Baccouri *et al.,* 2008). Regarding VCO, the data are still restricted. The present work aimed to elucidate characteristics of VCO recovered by different fruit maturities, in order to produce VCO with a good recovery yield and pleasant characteristics.

## 3.3 Objective

To study on the effect of coconut fruit maturity on properties of VCO

## 3.4 Materials and methods

### 3.4.1 Materials

Fresh coconut fruits at different maturities *i.e.,* 11, 12, and 13 months old were purchased from a local market in Hat Yai (Songkhla, Thailand). Alcalase, the protease from *Bacillus licheniformis,* with an activity of ≥ 5U/g (1 U corresponds to the amount of enzyme that sets free 1 µmol Folin-positive amino acids and peptide (as tyrosine) per min at pH 7.0 and 37 °C, using casein as a substrate), 2,2-bipyridine and Hydranal®-Coulomat AG-Oven were products of Sigma-Aldrich (St. Louis, MO, USA). Acetic acid, ethanol, toluene, chloroform, and methanol were purchased from J.T. Baker (Center Valley, USA.). Potassium iodide, potassium hydroxide, cyclohexane, and sodium carbonate were purchased from Ajax FineChem (Auckland, New Zealand). Folin Ciocalteau's and iodine monochloride were products of Fluka (St. Louis, MO, USA). All the chemicals and solvents used were of analytical grade.

### 3.4.2 Preparation of VCO

Coconut fruits with different maturities, *i.e*. 11, 12, and 13 months old referred as young– (YCO), intermediate– (ICO), and old coconut (OCO), respectively were used to prepare VCO via protease aided extraction followed the method of Prapun *et*

*al.* (2016). Briefly, coconut milk was firstly separated using a screw press (Fujica CM-SJ, Bangkok, Thailand). The grafted coconut meats were pressed for three times and separated milk was pooled together and thoroughly mixing for a few minutes. The coconut milk was destabilized using Alcalase at the concentrations of 0.3 % (w/w). Oil extraction was conducted at 60 °C for 120 min. The sample was then centrifuged at $15,000 \times g$ for 15 min, and the cream phase was further centrifuged at $15,000 \times g$ for 15 min to release clear oil.

### 3.4.3 Characterization of VCO

#### 3.4.3.1 Determination of peroxide value (PV)

PV was quantified according to the standard method of IUPAC (1992). The oil sample (5 g) was thoroughly mixed with a mixture of acetic acid:chloroform (3:2 v/v, 25 ml) and saturated KI solution (1 ml), before incubating in the dark for 1 h. After adding water (75 ml), the mixture was titrated with a standard solution of sodium thiosulfate (0.01 N) using a starch solution as an indicator.

#### 3.4.3.2 Determination of free fatty acid (FFA)

FFA was determined by a titration method (IUPAC, 1992). The oil (8 g) was mixed with ethanol (50 ml), before neutralizing with NaOH (0.01 N) using phenolphthalein as an indicator. FFA was expressed as a percentage of lauric acid.

#### 3.4.3.3 Determination of saponification value (SV)

SV measurement was carried out according to the method of IUPAC (1992). The oil (2 g) was mixed with KOH solution in ethanol (0.5 N, 25 ml), before distilling for 1 h. After cooling to room temperature, the mixture was titrated with HCl (0.5 N) using phenolphthalein as an indicator.

#### 3.4.3.4 Determination of iodine value (IV)

IV was determined by a standard method of IUPAC (1992). The oil (1 g) was thoroughly mixed with cyclohexane (15 ml) and Wijs solution (25 ml), before incubating in the dark for 1 h. After adding with KI solution (10 % w/v, 20 ml) and

water (150 ml), the mixture was titrated with a standard solution of sodium thiosulfate (0.1 N) using a starch solution as an indicator.

### 3.4.3.5 Determination of total phenolic content (TPC)

TPC was determined by Folin-Ciocalteu assay followed the method of Arslan *et al.* (2013) with a slight modification. Briefly, the oil (5 g) was mixed with a mixture of ethanol:water (80:20 v/v, 3 ml), before centrifuging at $5000 \times g$ for 5 min. This extraction was carried out for three times and all ethanolic extracts were combined, before evaporating until dryness using a rotary evaporator (Eyela N-1000, Tokyo, Japan). The dry matter was redispersed using a mixture of methanol:water (10:90 v/v, 1 ml), and added with water (8.2 ml) and Folin-Ciocalteau reagent (0.5 ml). The mixture was allowed to stand at room temperatures for 5 min, added with sodium carbonate solution (10 % w/v, 1 ml), and incubated at room temperature for 60 min. The absorbance at 765 nm was then read (UV-Vis Spectrophotometer, UV-1700, Shimadzu, Kyoto, Japan). TPC was calculated using a standard curve of gallic acid (0–100 µg/100 ml) and expressed as milligrams gallic acid equivalents (GAE) per kilogram of oil.

### 3.4.3.6 Determination of total tocopherols content

Tocopherol content present in the oils was measured according to the method described by Wong *et al.* (1998). The oil (1 g) was weighed accurately into 10 ml volumetric flask. Toluene (5 ml), 2,2-bipyridine (0.07 % w/v in 95 % ethanol, 3.5 ml) and $FeCl_2$ solution (0.2 % w/v in 95 % ethanol, 0.5 ml) were added to the sample. The solution was then made up to 10 ml using ethanol and allowed to stand at room temperature for 1 min, before reading the absorbance at 520 nm. Blank was prepared in a same manner, but oil was omitted. Tocopherol content was determined using a standard curve of α-tocopherol (0–250 µg/kg).

### 3.4.3.7 Determination of fatty acid composition

Fatty acid composition was examined according to the method of Chowdhury *et al.* (2007). The oil (50 µl) was added with KOH solution (0.5 N in methanol, 1 ml) and digested by stirring in a boiling water bath for 20 min. After

cooling to room temperature, the sample was added with a mixture of HCl:methanol (4:1 v/v, 0.4 ml), deionized water (2 ml) and petroleum ether (3 ml). The distinct upper layer of methyl ester was then separated carefully and dried by nitrogen gas. The sample was redispersed using chloroform (1 ml), before introducing to GC (Agilent technologies 7890A, Wilmington, USA) equipped with a flame ionization detector. Varian's capillary column was used (VF-5 ms, length 30 m $\times$ width 0.25 mm $\times$ particle size 0.25 μm; EZ-GRIP$^{TM}$, Wilmington USA). The column was conditioned at 180 °C for 2 h to attain thermal stability before use. The temperature condition was operated as following: holding at oven temperature of 150 °C for 5 min, increasing to 190 °C with a rate of 8 °C/min, increasing to 200 °C with a rate of 2 °C, and holding at 200 °C for 10 min.  Injection and detection temperatures were 250 °C. Nitrogen was used as a carrier gas with a flow rate of 20 ml/min.

### 3.4.3.8 Determination of moisture content

Moisture content in the oil was determined by coulometric Karl Fischer titration method using 831 Coulometric Karl Fischer and 728 Stirrer (Metrohm® Instruments, Canada, USA). Oil sample (1 ml) was accurately added into GL45 glass thread containing reagent for the reaction and stirred for 1 min. The titration curve was recorded on a strip chart recorder (Sam, 1998).

### 3.4.4 Statistical analysis

All experiments were carried out in triplicate with different three lots of oil, and the mean values with standard deviations were present. Completely Randomized Design was used. Statistical analysis of the data was performed by analysis of variance (ANOVA) using Duncan's multiple range test (SPSS for Windows, SPSS Inc., Chicago, IL, USA) at a 95 % confidence level.

## 3.5 Results and Discussion

The chemical composition of coconut meats at various age ranges was firstly determined, and the result was shown in **Table 11**

**Table 11** Chemical compositions of coconut meat at different maturities

| chemical compositions (% w/w, wet basis) | YCO | ICO | OCO |
|---|---|---|---|
| Moisture | $47.35\pm0.07^b$ | $49.50\pm0.1^a$ | $49.44\pm0.47^a$ |
| Ash | $1.07\pm0.01^b$ | $1.10\pm0.01^{ab}$ | $1.13\pm0.04^a$ |
| Protein | $8.45\pm0.10^b$ | $8.58\pm0.07^{ab}$ | $8.74\pm0.05^a$ |
| Fat | $33.29\pm0.49^b$ | $35.56\pm0.52^a$ | $30.30\pm0.29^c$ |
| Carbohydrate | $9.84\pm0.51^a$ | $7.26\pm0.52^c$ | $8.39\pm0.46^b$ |

Mean value ± standard deviation (n=3) were shown.

Letters within a same row indicate significant difference between means (P<0.05).

With different ripeness degrees, chemical composition of plants was dissimilar (Robertson *et al.,* 1978; Schittenhelm, 2008; Baccouri *et al.,* 2008). From **Table 11**, coconut meat was mainly consisted of moisture (*ca.* 47–49 %) and fat (*ca.* 30–35 %), irrespectively of age range. The highest fat content was observed for ICO (P<0.05), making it the most appropriate age range for coconut oil producing. This result was in accordance with the previous study (Sierra and Balleza, 1972). Jackson *et al.* (2004) reported the increased fat content of coconut with increased fruit maturity. At 10 months old, the endosperm growth is *ca.* 77.7 % with a lipid content of *ca.* 20.22 %, and after 12 months old the endosperm is fully growth with a lipid content of *ca.* 32.64 % (Jayalekshmy *et al.*, 1986). Accumulation of protein and ash with increasing coconut maturity was observed. Increasing of protein content might be associated with a development of endosperm from a watery jelly like to a harder white meat like (Purseglove, 1972).

The fatty acid compositions of the VCOs recovered by different fruit maturities were further determined as reported in **Table 12**.

**Table 12** Fatty acid compositions (% total fatty acids, TFAs) of the VCOs produced from coconut fruits with different maturities

| Fatty acids (%TFAs) | YCO | ICO | OCO | APCC standard* |
|---|---|---|---|---|
| Caprylic (C8:0) | $6.88\pm0.06^a$ | $5.81\pm0.71^b$ | $5.39\pm0.37^b$ | 5.00–10.00 |
| Capric (C10:0) | $6.99\pm0.16^a$ | $7.16\pm0.61^a$ | $7.15\pm0.23^a$ | 4.50–8.00 |
| Lauric (C12:0) | $51.52\pm0.39^a$ | $50.44\pm0.50^b$ | $51.81\pm0.13^a$ | 43.00–53.00 |
| Myristic (C14:0) | $17.56\pm0.53^a$ | $17.63\pm0.50^a$ | $17.49\pm0.52^a$ | 16.00–21.00 |
| Palmitic (C16:0) | $7.07\pm0.44^b$ | $8.02\pm0.08^a$ | $7.57\pm0.20^{ab}$ | 7.50–10.00 |
| Stearic (C18:0) | $2.38\pm0.19^b$ | $2.84\pm0.19^a$ | $2.78\pm0.13^a$ | 2.00–4.00 |
| Oleic (C18:1) | $6.53\pm0.25^a$ | $6.29\pm0.30^a$ | $6.30\pm0.09^a$ | 5.00–10.00 |
| Linoleic (C18:2) | $1.78\pm0.05^{ab}$ | $1.87\pm0.07^a$ | $1.51\pm0.24^b$ | 1.00–2.50 |
| Σ saturated fatty acids | $92.39\pm0.26^a$ | $91.87\pm0.42^{ab}$ | $92.20\pm0.73^a$ | |
| Σunsaturated fatty acids | $8.31\pm0.20^a$ | $8.16\pm0.25^{ab}$ | $7.81\pm0.20^b$ | |

Mean value ± standard deviation ($n$=3) were shown. Different letters indicate significant difference between means in a same row (P<0.05).

*The Asian Pacific Coconut Community (APCC) standards were shown in order for comparison (APCC, 2003).

In all samples, the most abundant fatty acids were medium chain fatty acids (MCFAs), especially for C12:0 with the content of 50.44–51.81% TFAs. Considering on unsaturated fatty acids, it was found that C18:1 was dominant with the content of 6.29–6.53 % TFAs. With different fruit maturities, a slight difference in fatty acid compositions of the VCO was evident. Comparing to the ICO and OCO, YCO provided the oil with the highest amounts of C8:0 (P<0.05). Regarding to health benefits of unsaturated fatty acids (Temime *et al.,* 2006), the present result implied a greater health benefit of the VCO produced from YCO than the other maturities (Reena and Lokesh, 2007; Raghavendra and Raghavarao, 2010). Maturity of plants could affect to fatty acid compositions of the extracted oils. For sunflower seed oil,

the content of C18:1 was 12 % at 7 days after the initiation of flowering (DAF), increased to 59.6 % at 14 DAF, and then diminished to 31.4 % by 56 DAF, whereas the C18:2 was present 48 % at 7 DAF, decreased to 23 % by 14 DAF, and turned to increase to 59.2 % by 56 DAF (Robertson *et al.,* 1978). Considering on extra virgin olive oil, the C18:1 content decreased gradually, whereas the C18:2 level increased as increase fruit ripened stage (Baccouri *et al.,* 2008). The diminishing in C16:0 content of extra virgin olive oil during ripening process was reported by Baccouri *et al.* (2008), supposed since a dilution effect (Gutiérrez *et al.,* 1999). Coconuts with different cultivars had their own unique characteristic including fatty acid compositions, that might be attributed to several factors such as location, age of fruits (Laurels *et al.,* 2000), and harvested time (Balleza and Sierra, 1976). Different fatty acid compositions were found for olive oils recovered from fruits with dissimilar cultivars (Temime *et al.*, 2006), expected due to different genetic factors and environmental conditions during fruit ripening process (Fedeli, 1977; Lavee and Wodner, 1995).

The chemical properties of the VCO recovered from fruits with different ripening stages were further characterized as shown in **Table 13**.

**Table 13** Chemical characteristics of the VCOs extracted from coconut fruits with different maturities

| Characteristics | YCO | ICO | OCO | APCC standard |
|---|---|---|---|---|
| PV<br>(milliEquiv. $O_2$/kg fat) | $0.11\pm0.03^c$ | $0.53\pm0.14^a$ | $0.37\pm0.08^b$ | Max 3 |
| FFA<br>(% lauric acid) | $0.09\pm0.01^b$ | $0.12\pm0.01^a$ | $0.09\pm0.01^b$ | Max 0.2 |
| SV<br>(mg KOH/g fat) | $255.82\pm3.05^a$ | $255.90\pm2.85^a$ | $254.12\pm3.26^a$ | 250–260 Min |
| IV<br>(g $I_2$/100g fat) | $7.94\pm0.03^a$ | $7.62\pm0.14^b$ | $7.36\pm0.04^c$ | 4.1–11 |
| Moisture content<br>(%) | $0.15\pm0.001^a$ | $0.15\pm0.001^a$ | $0.15\pm0.001^a$ | Max 0.1 |
| TPC<br>(mg GAE/kg) | $41.67\pm0.16^a$ | $35.02\pm0.10^b$ | $33.44\pm0.06^c$ | - |
| Total tocopherols<br>(mg/kg) | $0.024\pm0.0006^a$ | $0.003\pm0.0006^b$ | $0.003\pm0.0006^b$ | - |

Mean value $\pm$ standard deviation ($n$=3) were shown. Different letters indicate significant difference between means in a same row (P<0.05).

The Asian Pacific Coconut Community (APCC) standards were shown in order for comparison (APCC, 2003).

The lowest PV was found for the oil of YCO (P<0.05), suggesting to its better initial quality compared to the oils of ICO and OCO. The highest PV and FFA were observed for the oil of ICO (P<0.05), implying to a lower stability of the oil. This might be expected since a higher oil accumulation in the tissues of ICO, as indicated by the aforementioned result. For extra virgin olive oils, the FFA content tended to increase thoroughly with degree of maturity, which was explained by an undergoing of lipase activity and more sensitivity to pathogenic infections and mechanical damage of olive fruits with later maturity (Baccouri *et al.,* 2008). Coconut fruit has a hard shell to protect oil containing parts, so different oxidative behavior with maturity degree might be supposed. The highest IV was observed for the oil extracted from YCO (P<0.05), which was in agreement with its higher total unsaturated fatty acid

contents, compared to the oils of ICO and OCO (**Table 12**). Interestingly, the highest contents of nutritive microconstituents, involving both α-tocopherol and phenolic compounds, were found for the oils extracted from YCO ($P < 0.05$). Various compounds, *i.e.,* phenols, tocopherols, squalene, and pigments, were particularly higher in olive oils extracted from immature fruits compared to fully-ripened fruits (Gutiérrez *et al.,* 1999; Caponio *et al.,* 2001; Baccouri *et al.,* 2008). Tocopherols and phenolic compounds were responsible for antioxidant activity (Mateos *et al.,* 2003), so better oxidative stability tended to be observed for the tocopherol and phenolic rich oils (Gutiérrez *et al.,* 1999; Baccouri *et al.,* 2008). A decreasing in oleuropein content in olives with increasing fruit ripeness was reported, and attributed to phenolic degradation caused by the increased activity of hydrolytic enzymes, *e.g.,* esterase, at a later stage of maturity (Gutiérrez *et al.,* 1999; Ryan *et al.,* 2002). However, change in content of microconstituents with fruit maturity could be differed, depending on other factors such as growing condition and plant cultivar (Gutiérrez *et al.,* 1999; Baccouri *et al.,* 2008). It has been suggested that a greater degree of phenolic synthesis in fruit, and so in the extracted oil, related to enzyme activity in plant cells, such as L-phenylalanine ammonia-lyase whose activity was greater under higher water stress condition (Morello *et al.,* 2005).

## 3.6 Conclusion

Fruit maturity affected to chemical characteristics of the recovered VCO. The coconut at intermediate age stage contained the highest oil content than that observed at young and old ages. The VCO extracted from young fruits exhibited the best characteristics in both initial quality indicated by the lowest PV ($P < 0.05$) and nutritive value implied by the highest contents of tocopherols and phenolic compounds ($P < 0.05$). All of the observed parameters of the VCOs were within the limits of APCC standard, suggesting that the VCOs produced in the present work could be employed commercially.

# CHAPTER 4

# PHYSICOCHEMICAL STABILITY OF A MODEL
# VIRGIN COCONUT OIL-IN-WATER EMULSION

## 4.1 Abstract

Emulsion is generally found as a composition in various products involving foods, pharmaceuticals, cosmetics, and so on. Stability against both of phase separation and lipid oxidation is important to ensure the quality of emulsified products in several aspects, including sensory attribute, nutritive value, and safety. Oils employed as a dispersed phase to prepare oil-in-water (O/W) emulsion have a crucial role on physicochemical stability of the system. In this work, the colloidal and oxidative stabilities of emulsions prepared using different types of oil, *i.e*., virgin coconut oil (VCO) and soybean oil (SBO), were observed at various oil contents and storage times. Increasing of oil volume fraction and storage time generally affected to inferior dispersibility and oxidative stability of the emulsions. By employing VCO, the emulsions with a greater colloidal stability compared to the samples made from SBO could be prepared, especially at increased oil volume fraction. Better oxidative stability could be observed for the VCO emulsions than the SBO counterparts. Attributed to its health benefits, the present work suggests that VCO could be potentially used to prepare emulsified foods with desirable physicochemical stability and nutritive value that might be suitable for neutraceutical food production.

## 4.2 Introduction

Oil-in-water emulsion (O/W), defined as a system consisting of oil droplets dispersing through an aqueous media, is generally found as a composition in various food products, such as salad dressing, milk, sauces, infant formula, and so on (Siou *et al.,* 2014). To ensure consumer acceptability in emulsion product, stability against both phase separation and lipid oxidation has to be considered. Due to a presence of large interfacial area, emulsion is susceptible to lipid oxidation (Waraho *et al.,* 2011). Lipid oxidation has a great impact in lowering emulsion quality by rising off-flavor, reducing nutritive value of essential fatty acids and some vitamins, and also producing some

health risk compounds, *e.g.* free radicals and reactive aldehydes (Halliwell *et al.,* 1995). By using different oil types, the stability of emulsions was affected depending on fatty acid profiles and presence of microconstituents in the oils (Magnusson and Nilsson, 2011; Cornacchia and Roos, 2011). More pronounced drop aggregation was observed, when the oil with higher unsaturation degree was used than did the lower ones (Nor Hayati *et al.,* 2007). Therefore, emulsion with desirable physicochemical stability could be prepared by employing appropriate oil as a dispersed phase.

Presently, public awareness of functional food oil is increasing. VCO is widely consumed in tropical countries for thousand years. VCO is growing in popularity to be used as a functional ingredient for food processing, due to its richness in medium chain fatty acids (MCFAs) with a good digestibility (Che Man *et al.,* 1996) and bioactive microconstituents, *e.g.,* tocopherol, tocotrienol, and polyphenols (Dia *et al.,* 2005; Seneviratne *et al.,* 2009). Regarding to its higher polarity than those edible seed oils such as soybean and sunflower oils, VCO might exhibit interfacial activity and led to improve emulsion formability (Nor Hayati *et al.,* 2007). With high saturation degree and presence of natural compounds with antioxidant capacity, *e.g.,* tocopherol, tocotrienol, and polyphenols, VCO might provide emulsion with a good oxidative stability. Soybean oil (SBO) is commonly used to prepare various emulsified food products, such as dressings, sauces, and dips, in both household and industrial levels. Several microconstituents are naturally present in SBO, involving phospholipids and phytosterols, *e.g.,* tocopherol and β-sitosterol (Wang *et al.,* 1998). SBO mainly consists of unsaturated fatty acids, especially for linolenic acid with the content *ca.* 52 % of total fatty acids (Chaiyasit *et al.,* 2007), making SBO is prone to lipid oxidation (Chaiyasit *et al.,* 2000). This work aimed to elucidate physicochemical stability of O/W emulsion employing VCO as a dispersed phase in comparative with SBO. Colloidal and oxidative stabilities of the emulsions made from VCO or SBO at various oil volume fractions were investigated.

## 4.3 Objective

To elucidate the physicochemical stability of O/W emulsions produced from different types of oil, involving VCO and SBO

### 4.4 Materials and methods

#### 4.4.1 Materials

Fresh and mature coconuts (12 months old) were purchased from a local market in Hat Yai (Songkhla, Thailand). Refined SBO without exogenous antioxidant was provided by Lam Soon Public Company Limited (Bangkok, Thailand). Alcalase, the protease from *Bacillus licheniformis*, with an activity of $\geq 5U/g$ (1 U corresponds to the amount of enzyme that sets free 1 μmol Folin-positive amino acids and peptide (as tyrosine) per min at pH 7.0 and 37 °C, using casein as a substrate), cumene hydroperoxide, lyophilized bovine serum albumin (BSA; fraction V, minimum 96 % by agarose gel electrophoresis), malondialdehyde, thiobarbituric acid (TBA), and trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). $NaH_2PO_4.2H_2O$, $Na_2HPO_4.2H_2O$, ammonium thiocyanate, $BaCl_2$, $NaN_3$, sodium lauryl sulphate, and $FeSO_4.7H_2O$ were products of Ajax Fine Chem (Auckland, New Zealand). Isooctane, propanol, 1-butanol, and hydrochloric acid were bought from J.T. Baker (Center Valley, USA).

#### 4.4.2 Preparation of VCO

VCO was prepared by protease-aid extraction means following the method of Prapun *et al.* (2016). Briefly, coconut milk was firstly separated using a screw press (Fujica CM-SJ, Bangkok, Thailand). The grafted coconut meats were then pressed for three times, and the separated milk was thoroughly mixed for a few minutes. To separate oil, the coconut milk emulsion was destabilized using Alcalase. The enzyme was introduced to the coconut milk at the concentration of 0.3 %, w/w, and the extraction was carried out at 60 °C for 120 min. The sample was then centrifuged (Beckman Coulter Avanti JE, California, USA) at $15,000 \times g$ for 15 min, and the cream phase was further centrifuged at $15,000 \times g$ for 15 min to release clear oil.

#### 4.4.3 Preparation and characterization of O/W emulsion

VCO and SBO were employed as a dispersed phase to prepare O/W emulsion samples at various oil volume fractions (5–40 %, v/v). BSA was dissolved in 10 mM phosphate buffer pH 7 with a presence of 0.02 % $NaN_3$ to obtain the concentration of

0.5% (w/v), before mixing with the oil by homogenizer (IKA Labortechnik, Selangor, Malaysia) probe (S25N-18G-ST) at 19,000 rpm for 2 min. Three batches of emulsion samples were prepared separately and subjected to analyses.

### 4.4.3.1 Physical stability of the emulsions

- **Droplet size:** Mean diameter of oil drops was measured using a laser diffraction particle size analyzer (Brookhaven Instruments Ltd, Holtsville, New York). The volume mean diameter, $d_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3$, where $n_i$ is the number of droplets of the diameter of $d_i$, was reported.

- **Emulsifying activity index (EAI):** Freshly prepared emulsion (20 μl) was diluted with SDS solution (0.1 %, 5 ml), before observing the absorbance at 500 nm (UV–Vis Spectrophotometer Shimadzu UV-1700, Kyoto, Japan). EAI was calculated using the following equation (Guo and Mu, 2011):

$$EAI\,(m^2/g) = \frac{2 \times 2.303 \times A \times df}{c \times l \times (l - \phi) \times 10000}$$

where $A$ is the absorbance at 500 nm, $df$ the dilution factor, $c$ the initial protein concentration in the emulsion (g/ml), $l$ the optical path (0.01 m), and $\phi$ the oil volume fraction.

- **Creaming index (CI):** The emulsion (8 ml) was transferred to a test tube tightly sealed with a plastic cap, before centrifuging at $1,950 \times g$ for 20 min. CI was calculated:

$$CI(\%) = \left(H_C / H_T\right) \times 100$$

where $H_C$ and $H_T$ are the height of the cream layer and the total height of emulsion, respectively (Sun and Gunasekaran, 2009).

### 4.4.3.2 Oxidative stability of the emulsions

The emulsion sample was transferred to an amber bottle with a screw cap and kept at 50±2 °C for 4 weeks in the dark. Progressive of lipid oxidation was periodically monitored.

**- Peroxide value (PV):** The emulsion (0.6 g) was mixed with a mixture of isooctane:propanol (3:1 v/v, 3 ml), before centrifuge at $1,950 \times g$ for 2 min. A clear upper layer (200 µl) was taken out to react with a mixture of methanol:1-butanol (2:1 v/v, 2.8 ml), ammonium thiocyanate (3.97 M, 15 µL), and ferrous iron solution containing an equal volume of 0.132 M $BaCl_2$ and 0.144 M $FeSO_4.7H_2O$ (15 µL). After incubation at room temperature for 20 min, the absorbance at 510 nm was determined. PV was quantified using a standard curve of cumene hydroperoxide (0–250 mg/kg sample) and expressed as mg cumene hydroperoxide equivalent/kg sample (Hannah and Casimir, 2004).

**- Thiobarbituric acid reactive substances (TBARs)**: The emulsion (1 ml) was mixed with TBA reagent (5 ml) consisting of trichloroacetic acid (15 % w/v) and thiobarbituric acid (0.375 % w/v) in HCl (0.25 M). After heating at 100 °C for 10 min, the mixture was allowed to cool to room temperature. The sample was then centrifuged at $7,000 \times g$ for 10 min, and the absorbance at 532 nm was read. TBARs content was determined using a standard curve of malondialdehyde (0–100 mg/kg sample) and expressed as mg malondialdehyde equiv/kg sample (Hannah and Casimir, 2004).

### 4.4.4 Statistical analysis

All experiments were carried out in triplicate, and the mean values with standard deviations were presented. Completely randomized design was used. Statistical analysis of the data was performed by analysis of variance (ANOVA) using Duncan's multiple range test (SPSS for Windows, SPSS Inc., Chicago, IL, USA) at a 95 % confidence level.

## 4.5 Results and discussion

### 4.5.1 Colloidal stability of the emulsions

Initial size of the emulsions made from VCO and SBO at various oil contents was investigated and the result was shown in **Figure 6**. Increase oil volume fraction thoroughly affected to increase $d_{4,3}$ (P<0.05) of the emulsions. Under a fixed emulsification circumstance, *i.e.*, homogenizing condition and emulsifier concentration

in the present work, larger generated interfacial areas could be expected for the emulsions containing higher oil content, so a present emulsifier might not be able to totally cover the interfacial areas (McClements and Decker, 2000). With a presence of bare interfaces, drop aggregation could be enhanced, resulting in a bigger droplet size (Dickinson, 2015). By using dissimilar types of dispersed phase, emulsion size was affected. At a same oil content, the smaller oil drops could be observed for the samples made from VCO compared to the SBO counterparts (P<0.05). This might be attributed to a different characteristic of the used oils. The predominant fatty acids of VCO are MCFAs, especially for lauric and myristic acids, whereas SBO mainly consists of long chain fatty acids, especially for linoleic acid (Chowdhury *et al.,* 2007). Greater hydrophobicity of SBO might cause a higher tension at the oil-water interfaces, resulting in bigger produced oil drops (Muhannad and Bernd, 1998; Driscoll *et al.,* 2001). Moreover, it has been suggested that a bigger initial drop size was tended to be observed when the oils with higher viscosity was used, because more intense disruption force was needed to deform oil drops during emulsification process. The viscosities of soybean and coconut oils were reported as 31.8 and 28 cP, respectively (Hossein *et al.,* 1992). Interestingly, polydispersities of the concentrated emulsions containing oil contents up to 30 % made from VCO were lowered than those of the SBO emulsions at a corresponding oil content. Polydispersity is one of the factors indicating colloidal stability of emulsion, in which the lower polydispersity the greater emulsion dispersibility (Laura *et al.,* 2015). Therefore, the present work suggested that VCO could be used to prepare concentrated emulsion with a greater stability than did SBO.
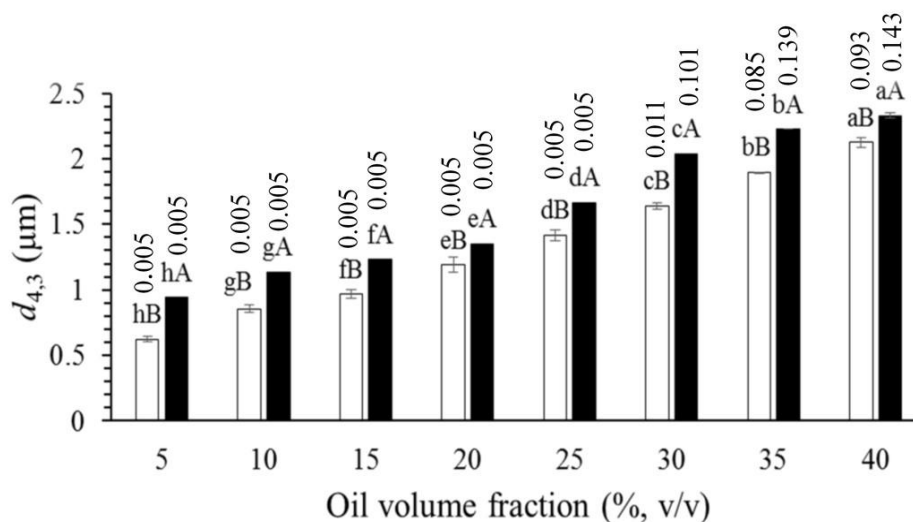
**Figure 6** Mean diameters ($d_{4,3}$) of the emulsions made from VCO (open bar) and SBO (closed bar) at various oil volume fractions. The numbers in parentheses represent polydispersity index of the samples. Mean value ± standard deviation ($n$=3) were shown.

Different lower letters indicate significant difference between means in a same type of oil (P<0.05).

Different upper letters indicate significant difference between means in a same oil volume fraction (P<0.05).



**Figure 7** Emulsifying activity index (EAI) of the emulsions made from VCO (open bar) and SBO (closed bar) at various oil volume fractions. Mean value ± standard deviation ($n$=3) were shown.

Different lower letters indicate significant difference between means in a same type of oil (P<0.05).

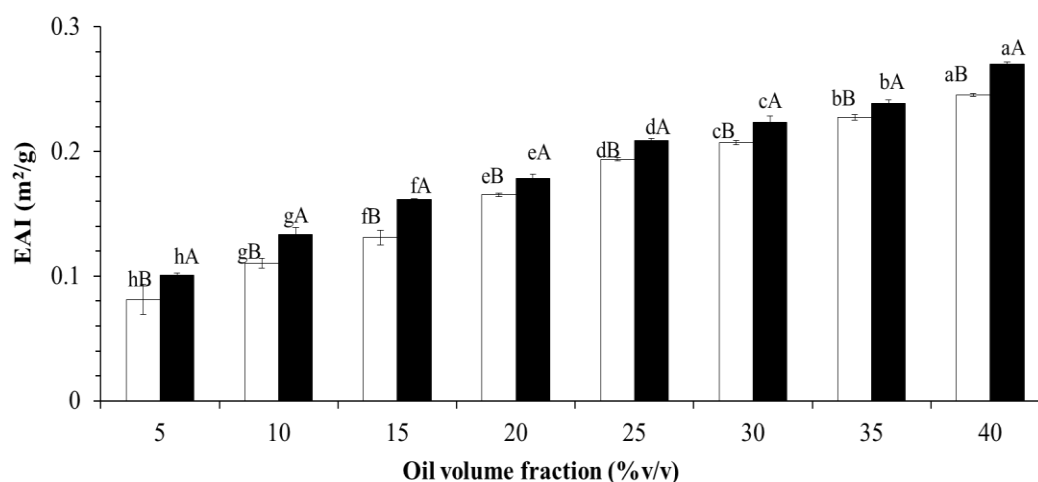Different upper letters indicate significant difference between means in a same oil volume fraction (P<0.05).

EAI of the emulsions was depicted in **Figure 7**. Increase oil content thoroughly affected to increase EAI of the emulsions, irrespectively of oil types (P<0.05). EAI is a factor indicating interfacial areas that can be covered by a weight of protein and can be used to imply how fast proteins are adsorbed to interfacial areas (Webb *et al.,* 2002). A larger interfacial area of the emulsions with higher oil contents might allow more conformational changes of the proteins through surface denaturation process, thereby resulting in a faster protein adsorption (Cheetangdee, 2014). By employing SBO as a dispersed phase, higher EAI could be observed than did VCO, irrespectively of oil content (P<0.05). During emulsification process, greater extension of protein's surface denaturation could be expected when the oils with higher hydrophobicity were employed, attributed to a higher hydrophobic driving force for protein unfolding (McClements, 2004). More pronounced protein unfolding might promote protein adsorption, so larger interfacial areas could be covered by an amount of proteins. Consequently, higher EAI could be expected when SBO was employed for emulsion preparation than did VCO.

Further, colloidal stability of the emulsions was monitored under an accelerated condition of centrifugal force and expressed as CI in **Figure 8**. Increase oil volume fraction significantly affected to increase CI (P<0.05), suggesting to an inferior emulsion dispersibility. This could be supposed since a larger drop size of the emulsions containing higher oil content as reported in **Figure 6.** Impaired colloidal stability of the emulsion with larger sized oil drops has been previously confirmed (Dickinson and Golding, 1997; Laura *et al.,* 2015). Emulsion stability decreased with increased oil volume fraction, because of the increase in packing fraction of oil droplets. This led to increase a collision frequency between dispersed drops, thereby accelerating drop aggregation (Dickinson and Golding, 1997). Network formation of flocculated oil drops was enhanced by increasing oil content of the emulsions, especially up to 40 %, resulting in a rapid cream forming (Guo and Mu, 2011). Considering on the effect of oil type, significant difference was not observed for the CI of the SBO and VCO emulsions at all observed oil contents (P>0.05).
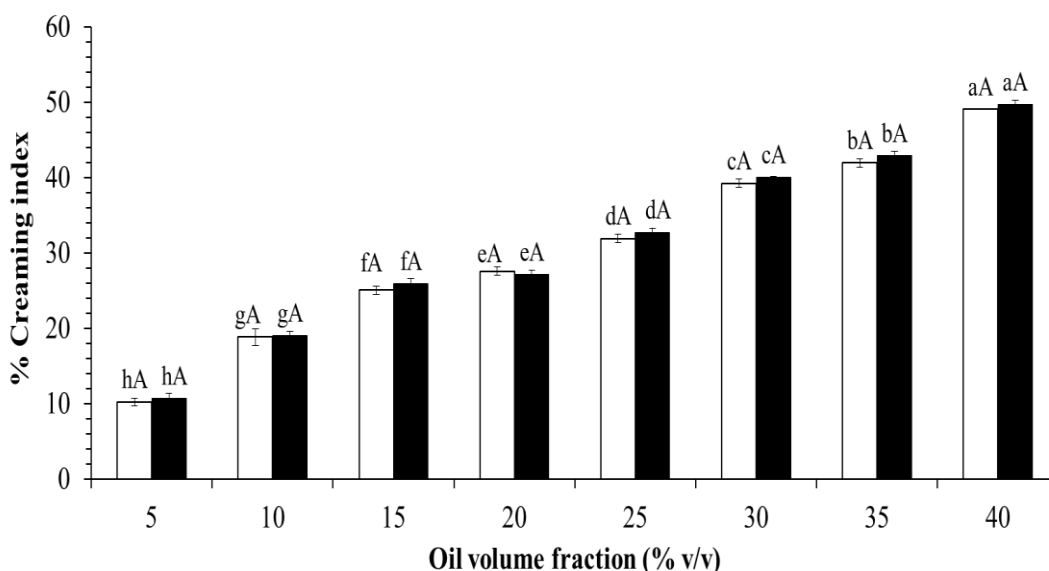
**Figure 8** Creaming index (CI) of the emulsions made from VCO (open bar) and SBO
(closed bar) at various oil volume fractions. Mean value ± standard deviation
($n$=3) were shown.

Different lower letters indicate significant difference between means in a same type of oil (P<0.05).

Different upper letters indicate significant difference between means in a same oil volume fraction
(P<0.05).

Long term stability of the emulsions was further elucidated measuring the
percentage of oil droplet size increasing at different storage times: % increase
$d_{4,3}$=100×[($d_{4,3}(t)$-$d_{4,3}(0)$) /$d_{4,3}(0)$], where $d_{4,3}(0)$ and $d_{4,3}(t)$ are the mean diameters of
oil drops measured at initial and at time $t$, respectively (Rao and McClements, 2012).
**Figure 9** illustrates % increase $d_{4,3}$ of the emulsions containing different oil contents
after storing for 2 and 4 weeks. Increase oil volume fraction led to inferior emulsion
dispersibility, as implied by a higher % increase $d_{4,3}$ of the emulsions with higher oil
contents. This behavior was in accordance with the previous results reported in
**Figure 6** and **8**. Upon prolonging a storage time, drop aggregation could be enhanced
via a driving of several interparticle attractive forces such as *van Der* Waals and
hydrophobic interactions (McClements, 2012). The % increased $d_{4,3}$ of the emulsions
generally seemed to independent with oil types (P>0.05). Nonetheless, after 4 weeks of
storage, a significantly lower drop size increasing was found for the VCO emulsions
compared to the SBO counterparts, when the samples contained oil contents up to 35
% (P<0.05). This result indicated an effective ability of VCO to preserve long term

dispersibility of the concentrated emulsion, in which an inferior colloidal stability could be generally expected (McClements, 2012).
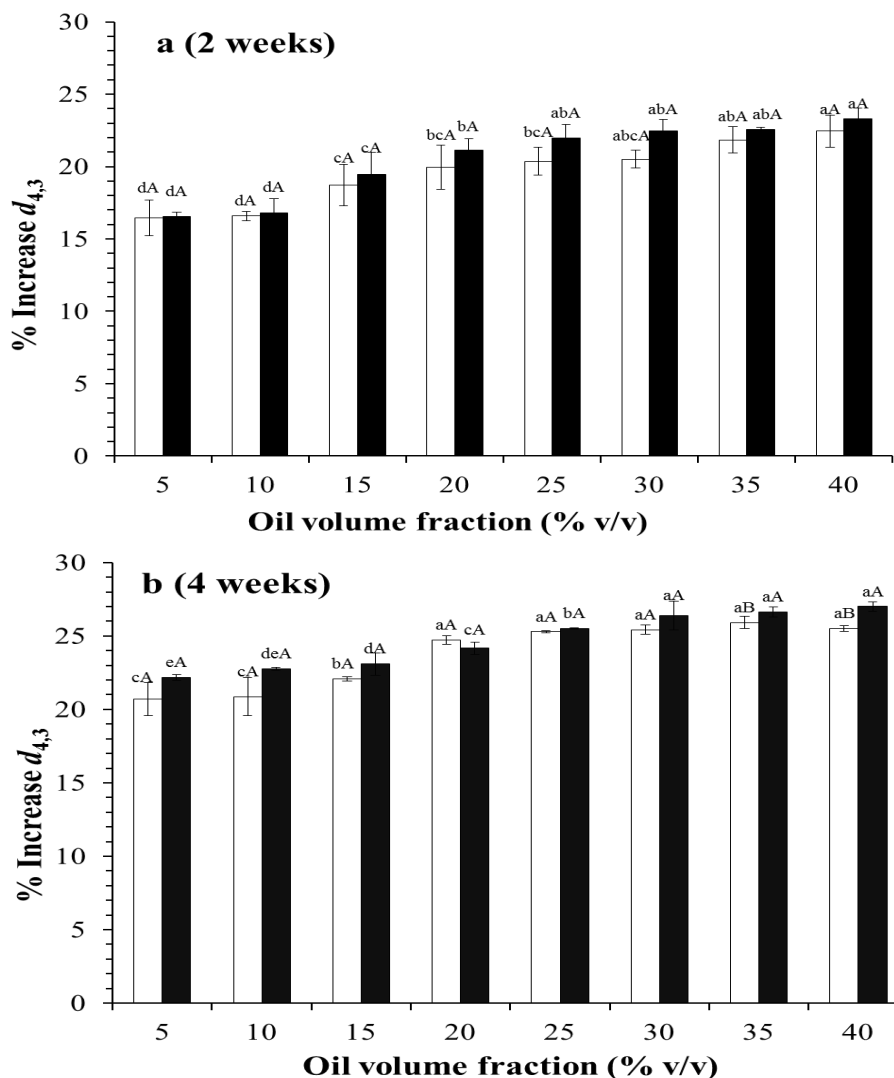


**Figure 9** Percentage of $d_{4,3}$ increasing of the emulsions made from VCO (open bar) and SBO (closed bar) at various oil volume fractions. The emulsions were kept for (a) 2 and (b) 4 weeks. Mean value ± standard deviation (n=3) were shown.

In each subfigure, different lower letters indicate significant difference between means in a same type of oil (P<0.05).

In each subfigure, different upper letters indicate significant difference between means in a same oil volume fraction (P<0.05).

**4.5.2 Oxidative stability of the emulsions**

To evaluate degree of lipid oxidation occurring in the emulsions, PV and TBARs were monitored along a period of 4 weeks, and the results were shown in **Table 14 and 15** respectively.

With a presence of large interfacial areas, emulsion is prone to lipid oxidation (McClements and Decker, 2000; Waraho *et al.,* 2011). Characteristics of dispersed drops, *i.e.,* size, concentration, and polydispersity, could influence oxidative degree in emulsion system (McClements and Decker, 2000; Waraho *et al.,* 2011). From **Table 14** and **15**, lower oxidative degree as implied by lower PV and TBARs could be observed when the emulsions contained higher oil volume fractions. Higher oxidative degree was coincident with a smaller drop size of the emulsions with lower oil content (see **Figure 6**). As a result of smaller size of dispersed oil drops, lipid oxidation in emulsion system could be accelerated, because of a higher ratio of surface area/oil volume, resulting in a greater exposure area of oil phase to water soluble pro-oxidants (McClements and Decker, 2000). This tendency was in agreement with the study of Osborn and Akoh (2004) reported that the emulsion containing 10 % oil content had a markedly higher hydroperoxide amount than the sample with 30 % oil content. Accumulation of oxidation products in term of both PV and TBARs was increased with a prolonged storage time ($P<0.05$). PV (TBARs) of the SBO emulsion containing 5 % oil content increased from $8.65\pm0.33$ ($1.32\pm0.01$) in the first week to $77.99\pm0.69$ mg hydroperoxide equivalent/kg sample ($17.53\pm0.02$ mg malondialdehyde equivalent/kg sample) after 4 weeks of storage. A significantly increased PV of canola oil emulsion along a period of 15 days was also reported (Osborn and Akoh, 2004).

At a corresponding oil content, higher oxidative degree could be observed for the SBO emulsions compared to the VCO counterparts as implied by a remarkably higher PV and TBARs of the former emulsions ($P<0.05$). Although the larger interfacial areas could be supposed for the VCO emulsions due to their smaller sized oil drops (see **Figure 6**), greater oxidative stability of VCO emulsions might be attributed to higher saturation degree of its fatty acid component. Susceptibility against lipid oxidation of unsaturated fatty acids abundantly present in SBO has been confirmed (McClements and Decker, 2000). Considering in a bulk model, accumulation of oxidized compounds

was greater as the unsaturation degree of oil increased (Martín *et al.,* 2004). In emulsion model, the characteristics of the oil-water interfaces had a crucial role in oxidative stability of the system (Chaiyasit *et al.,* 2000; Donnelly *et al.,* 1998). Unsaturated fatty acids might exhibit a strong pro-oxidant effect in emulsion model: Oleic and linoleic acids could induce a significant reduction in negative charges of the dispersed oil drops, that led to enhance ability of aqueous soluble transition metal ions to attack surfaces of oil drops, thereby promoting degree of lipid oxidation (Waraho *et al.,* 2011). The higher oxidative degree of SBO emulsions, moreover, might be supposed due to longer storage time of SBO than did VCO.

**Table 14** PV of the emulsions made from VCO and SBO at various oil contents and storage times

| Oil volume fraction (%) | VCO | | | SBO | | |
|---|---|---|---|---|---|---|
| | Week0 | Week2 | Week4 | Week0 | Week2 | Week4 |
| 5 | $1.80\pm0.12^{aC}$ | $5.59\pm0.93^{aB}$ | $11.39\pm1.25^{aA}$ | $8.65\pm0.33^{aC}$ | $60.43\pm0.65^{aB}$ | $77.99\pm0.69^{aA}$ |
| 10 | $1.67\pm0.21^{aC}$ | $5.06\pm1.11^{aB}$ | $10.48\pm0.52^{aA}$ | $7.99\pm0.89^{abC}$ | $59.45\pm0.26^{aB}$ | $74.21\pm0.87^{abA}$ |
| 15 | $1.32\pm0.12^{bC}$ | $5.70\pm0.49^{aB}$ | $10.99\pm1.09^{aA}$ | $7.53\pm0.49^{abcC}$ | $53.86\pm0.18^{bB}$ | $73.38\pm0.30^{bcA}$ |
| 20 | $0.67\pm0.10^{cC}$ | $5.10\pm0.28^{aB}$ | $7.88\pm1.06^{bA}$ | $7.33\pm0.39^{bcC}$ | $53.43\pm0.46^{bB}$ | $72.82\pm0.61^{bcA}$ |
| 25 | $0.65\pm0.25^{cC}$ | $3.86\pm0.59^{bB}$ | $5.87\pm1.50^{cA}$ | $6.66\pm0.54^{cC}$ | $37.11\pm0.41^{cB}$ | $73.00\pm0.19^{bcA}$ |
| 30 | $0.17\pm0.01^{dC}$ | $3.23\pm0.07^{bcB}$ | $4.18\pm0.05^{cdA}$ | $4.06\pm0.54^{dc}$ | $27.60\pm0.28^{dB}$ | $63.83\pm3.22^{eA}$ |
| 35 | $1.08\pm0.24^{bC}$ | $2.38\pm0.30^{cB}$ | $4.21\pm0.89^{cdA}$ | $3.61\pm0.61^{dC}$ | $27.91\pm0.49^{dB}$ | $69.96\pm3.30^{cdA}$ |
| 40 | $0.65\pm0.32^{cC}$ | $2.97\pm0.38^{bcB}$ | $3.79\pm0.08^{dA}$ | $4.14\pm1.05^{dC}$ | $17.23\pm4.83^{eB}$ | $67.01\pm3.98^{deA}$ |

In each parameter, different lower letters indicate significant difference between means in a same column (P<0.05).

In each parameter, different upper letters in a same oil type indicate significant difference between means in a same row (P<0.05).

A same oil fraction and storage time indicate significant difference between means (P<0.05).

64

**Table 15** TBARs of the emulsions made from VCO and SBO at various oil contents and storage times

| Oil volume fraction (%) | VCO | | | SBO | | |
|---|---|---|---|---|---|---|
| | Week0 | Week2 | Week4 | Week0 | Week2 | Week4 |
| 5 | $0.55\pm0.02^{a}$ | $0.65\pm0.11^{abB}$ | $1.16\pm0.04^{aA}$ | $1.32\pm0.01^{aC}$ | $7.72\pm0.08^{aB}$ | $17.53\pm0.02^{aA}$ |
| 10 | $0.45\pm0.01^{bC}$ | $0.69\pm0.04^{aB}$ | $0.98\pm0.04^{bA}$ | $1.13\pm0.03^{bC}$ | $7.62\pm0.03^{abB}$ | $16.76\pm0.10^{bA}$ |
| 15 | $0.40\pm0.01^{cC}$ | $0.56\pm0.02^{bcB}$ | $0.84\pm0.10^{cA}$ | $1.02\pm0.09^{cC}$ | $7.37\pm0.10^{abB}$ | $16.08\pm0.08^{cA}$ |
| 20 | $0.38\pm0.01^{cC}$ | $0.51\pm0.07^{cdB}$ | $0.70\pm0.01^{dA}$ | $1.04\pm0.03^{cC}$ | $7.23\pm0.32^{bB}$ | $15.69\pm0.20^{dA}$ |
| 25 | $0.36\pm0.01^{cC}$ | $0.42\pm0.05^{deB}$ | $0.68\pm0.02^{dA}$ | $0.86\pm0.02^{dC}$ | $5.60\pm0.23^{cB}$ | $14.17\pm0.01^{eA}$ |
| 30 | $0.23\pm0.05^{dC}$ | $0.31\pm0.01^{fB}$ | $0.64\pm0.01^{dA}$ | $0.84\pm0.04^{dC}$ | $4.89\pm0.45^{dB}$ | $13.02\pm0.12^{fA}$ |
| 35 | $0.13\pm0.04^{eC}$ | $0.30\pm0.03^{fB}$ | $0.55\pm0.05^{eA}$ | $0.53\pm0.01^{eC}$ | $4.55\pm0.18^{dB}$ | $11.53\pm0.14^{gA}$ |
| 40 | $0.07\pm0.01^{fC}$ | $0.41\pm0.06^{eB}$ | $0.63\pm0.01^{deA}$ | $0.22\pm0.06^{fC}$ | $1.40\pm0.30^{eB}$ | $7.22\pm0.01^{hA}$ |

In each parameter, different lower letters indicate significant difference between means in a same column (P<0.05).

In each parameter, different upper letters in a same oil type indicate significant difference between means in a same row (P<0.05).

A same oil fraction and storage time indicate significant difference between means (P<0.05).

**4.6 Conclusion**

By employing different oil types, physicochemical stability of emulsion was affected. Increase oil volume fraction resulted in lowered emulsion dispersibility. By using VCO, the emulsions with greater colloidal stability could be prepared, especially at high oil content, *i.e.,* up to 30 % in the present work. Moreover, the emulsions with a significantly greater oxidative stability could be prepared using VCO, as suggested by lower PV and TBARs contents of the VCO emulsions than those observed for SBO counterparts along a storage of 4 weeks. Regarding to its health benefits, therefore, VCO might be a promising candidate used to prepare high oil containing emulsified food products with desirable physicochemical stability and nutritive value.

**CHAPTER 5**

**PHYSICOCHEMICAL STABILITY OF VIRGIN COCONUT OIL**

**SALAD DRESSING STABILIZED BY TAPIOCA MALTODEXTRIN**

## 5.1 Abstract

Salad dressing is a widely consumed oil containing product. To ensure acceptability of the product, stability against both of phase separation and lipid oxidation has to be considered. In this work, physicochemical properties of the dressing prepared from virgin coconut oil (VCO) were investigated by comparing with soybean oil (SBO). Tapioca maltodextrin (TMD) was employed as a stabilizer and its concentration effect on the characteristics of dressing samples was examined. Comparable dispersibility of the dressings made from VCO and SBO was observed. Incorporation of TMD, especially at increased concentration could enhance colloidal stability of the dressings, as suggested by the lowered increasing of oil droplet size and creaming rate through 8 weeks of storage. This could be supposed due to increase viscosity of the dressings by TMD adding. Better oxidative stability could be observed for the VCO dressings than the SBO counterparts, postulated due to higher phenolic contents and saturation degree of composited fatty acids of VCO. Considering on sensory evaluation, the dressing made from VCO exhibited appreciable acceptability, especially when TMD was applied at 3 %. The present work suggests that VCO could be used to produce salad dressing with desirable physicochemical stability and high phenolic content, especially with TMD incorporating.

## 5.2 Introduction

Salad dressing is oil-in-water emulsion (O/W) that is widely used to enhance attractiveness and tastiness in various food products. To ensure acceptability of the dressing product, colloidal and oxidative stability has to be concerned. Dressing is susceptible to phase separation and oxidative deterioration since it always contains high oil content of *ca.* 40–60 % (Ma *et al.,* 2013). Maltodextrins, the biopolymer of D-glucose subunits linked linearly with α (1,4)-glycosidic bonds and with α (1,6)-glycosidic bonds for branching, are widely used as a stabilizer to enhance desirable texture and stability of emulsified food products (Hogan *et al.,* 2004; Klinkesorn *et al.,*

2004; McClements, 2004; Udomrati *et al.,* 2013). Tapioca is one of the important agricultural products of Thailand with the harvested amount of 33 million tons in 2015 (Thai tapioca trade association, 2015). Ability of tapioca maltodextrin (TMD) to enhance stability of the model emulsion has been reported. Udomrati *et al.* (2013) found that by using TMD with dextrose equivalent (DE) of 9 at the concentration of 35 %, viscosity of the model SBO emulsion could be controlled appropriately, resulting in lowered flocculation and coalescence degree of dispersed oil droplets. Considering on the effect of TMD on stability of emulsified foods, however, the data are still restricted.

Virgin coconut oil (VCO) is growing in popularity to be used as a functional ingredient for food processing, regarded due to its richness in bioactive compounds such as vitamin E and polyphenol (Kapila *et al.,* 2008, 2009), as well as good digestibility of medium chain fatty acids (Che Man *et al.,* 1996). VCO consisted predominantly of saturated fatty acids, especially for lauric acid with the content of 45–56 % of total fatty acids (Gopala *et al.,* 2010). With its high saturation degree, VCO is stable to oxidative reaction that might be useful for extending chemical stability of the emulsified products. Considering on physical stability, it has been suggested that the oil with higher saturation degree could be used to prepare the emulsion with greater dispersibility than did the lower ones (Chaiyasit *et al.*, 2000). By selecting different oils as a dispersed phase, physicochemical properties of the emulsified products were affected. In our previous study, the model emulsions containing high oil content, *i.e.,* up to 30 % with a greater colloidal and oxidative stability could be prepared by using VCO compared to the counterparts made from soybean oil (SBO). This work aimed to elucidate physicochemical stability of the salad dressing made from VCO as affected by TMD incorporation at various concentrations. In order for comparison, SBO was also employed to prepare dressing sample. The dressing samples were stored at various storage times, and their characteristics involving dispersibility, oxidative stability and rheological property were examined.

**5.3 Objective**

    **-** To develop salad dressing recipe with a desirable stability using VCO as a dispersed phase

    - To study on the effect of TMD DDING on characteristics and stability of salad dressing product

**5.4 Materials and methods**

    **5.4.1 Materials**

    Fresh and mature coconuts (12 months old), egg yolk, sugar, salt, vinegar, and mustard were purchased from a local market in Hat Yai (Songkhla, Thailand). Refined SBO without exogenous antioxidant was provided by Lam Soon Public Company Limited (Bangkok, Thailand). TMD with DE of 9 was supported by Corn Products Public Company Limited (Bangkok, Thailand). Alcalase, the protease from *Bacillus licheniformis*, with an activity of $\geq 5$U/g (1 U corresponds to the amount of enzyme that sets free 1 $\mu$molFolin-positive amino acids and peptide (as tyrosine) per min at pH 7.0 and 37 °C, using casein as a substrate), cumene hydroperoxide, malondialdehyde, thiobarbituric acid, *p*-anisidine, and trichloroacetic acid were products of Sigma-Aldrich (St. Louis, MO, USA). Ammonium thiocyanate, $BaCl_2$, and $FeSO_4.7H_2O$ were procured from Ajax FineChem (Auckland, New Zealand). Isooctane, propanol, 1-butanol, and hydrochloric acid were purchased from J.T. Baker (Center Valley, USA).

    **5.4.2 Preparation of VCO**

    VCO was prepared using protease aided extraction followed the method of Prapun *et al.* (2016). Briefly, the grafted coconut meats were pressed using a screw press (Fujica CM-SJ, Bangkok, Thailand) for three times, and the separated milk was thoroughly mixed together for a few minutes. To separate oil, Alcalase was introduced to the coconut milk at the concentration of 0.3 %, w/w, before conducting oil extraction at 60 °C for 120 min. The sample was then centrifuged (Beckman Coulter Avanti JE, California, USA) at $15,000 \times g$ for 15 min, and the cream phase was further centrifuged at $15,000 \times g$ for 15 min to release clear oil.

### 5.4.3 Salad dressing preparation

The dressing was produced in a laboratory scale at a total weight of 500 g, followed the method of Zhen and Joyce (2013). Firstly, a premix was prepared using vinegar (18 %), egg yolk (6 %), sugar (23 %), salt (1.8 %), mustard (3 %), potassium-sorbate (0.1 %), and TMD at various concentrations (0.5, 1.5 and 3 %). The oil phase (48 %) was added drop wise to the aqueous phase. Blending was performed using a blender (Tefal BL1161AD, Jakarta, Indonesia) for 4 min at room temperature. The dressings were transferred into a glass bottle and stored in a condition similar to those in a convenience store by placing in a shelf unit at ambient temperature with illumination for 12 h/day. Three batches of the dressings were prepared separately. The dressing samples were kept for 2 months and subjected to analyses.

### 5.4.4 Physical characteristics of the dressings

- **Droplet size:** Mean diameters of oil drops were measured using a laser diffraction particle size analyzer (Brookhaven Instruments Ltd, Holtsville, New York). The volume mean diameter, $d_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3$, where $n_i$ is the number of droplets of the diameter of $d_i$, was reported.

- **Creaming index (CI):** The dressing (10 g) was transferred to a test tube tightly sealed with a plastic cap, before centrifugation at $7,690 \times g$ for 25 min. CI was calculated as follows;

$$CI(\%) = \left( H_C / H_T \right) \times 100$$

where $H_C$ and $H_T$ are the height of the cream layer and the total height of emulsion, respectively (Sun and Gunasekaran, 2009).

- **Color measurement:** Color of the dressing samples was measured using a colormeter (Hunter Lab ColorFlex, Hunter Associates Laboratory, Virginia, USA). A fixed amount of dressing was poured into a measuring cup, surrounded with a black paper strip. Color parameters including $L^*$ representing lightness to darkness (0=black and 100=white), $a^*$ exhibiting redness (+) to greenness (-), and $b^*$ indicating yellowness (+) to blueness (-) were measured. Total change in color ($\Delta E$) with storage time was calculated using the following equations (Zhen *et al*., 2013):

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

where $\Delta L^*$, $\Delta a^*$, $\Delta b^*$ are the difference on $L^*$, $a^*$, and $b^*$ value at the initial time and at time t, respectively.

### 5.4.5 Chemical characteristics of the dressings

**- pH:** The dressing sample (1 g) was mixed with DI water (10 ml), before measuring pH using a pH meter (FEP20, Mettier-Toiedo AG, Schwerzenbach, Switzerland) (Adebayo *et al.,* 2010).

- **Total phenolic content (TPC):** The dressing sample (8 g) was mixed with acidified acetone consisted of 70 % acetone and 0.1 % HCl at a ratio of 4:1 (v/v) (15 ml). The mixture was then allowed to stand at room temperate for overnight, before filtering through Whatman filter paper (No.1). The supernatant (10 ml) was concentrated by a rotary vacuum evaporator (Eyela N-1000, Tokyo, Japan) at 45 °C until dryness. The dry matter was redispersed using a mixture of methanol:water (10:90 v/v, 1 ml), before adding with water (8.2 ml) and Folin-Ciocalteau reagent (0.5 ml). The mixture was allowed to stand at room temperatures for 5 min, before reacting with sodium carbonate solution (10 % w/v, 1 ml). After incubating at room temperature for 60 min, the absorbance at 765 nm (UV-Vis Spectrophotometer, UV-1700, Shimadzu, Kyoto, Japan) was read. TPC was calculated using a standard curve of gallic acid (0–100 µg/100 ml) and expressed as mg gallic acid equivalents (GAE) per kilogram of sample (Tseng and Zhao, 2013).

The salad dressings were transferred to amber bottles and kept at 50±2°C for 8 weeks in the dark. Progressive of lipid oxidation was periodically monitored measuring peroxide value (PV), thiobarbituric acid reactive substances (TBA) and para-anisidine value (*p*-AnV).

**- PV determination:** The dressing (0.6 g) was mixed with a mixture of isooctane:propanol (3:1 v/v, 3 ml), before centrifugation at $1,950 \times g$ for 2 min. A clear upper layer (200 µl) was taken out to react with a mixture of methanol:1-butanol (2:1 v/v, 2.8 ml), ammonium thiocyanate (3.97 M, 15 µL), and ferrous iron solution (15 µL) containing an equal volume of 0.132 M $BaCl_2$ and 0.144 M $FeSO_4.7H_2O$. After incubation at room temperature for 20 min, the absorbance at 510 nm was determined.

PV was quantified using a standard curve of cumene hydroperoxide (0–250 mg/kg sample) and expressed as mg hydroperoxide equivalent/kg sample (Hannah and Casimir, 2004).

**- TBA determination:** The dressing (10 g) was mixed with distilled water (97.5 ml), 4 N HCl (2.5 ml) and antifoaming. The mixture was distillated, and the received volume of 50 ml was transferred to react with 2-thiobarbituric acid (TBA) reagent consisted of 0.28 % TBA in 90 % acetic acid (5 ml). The mixture was heated in a boiling water at 100 ℃ for 35 min, and cooled in an ice bath for 10 min. The absorbance at 532 nm was then read. The content of TBA was quantified using a standard curve of malondialdehyde (0–100 mg/kg sample) and expressed as mg malondialdehyde equivalent/kg sample (Rossell, 1994).

**- *p*-AnV determination:** Briefly, the dressing sample (2 g) was added into a 25 ml volumetric flask and made up to volume with isooctane. After mixing thoroughly, the sample was transferred to a 50 ml centrifuge tube and vortexed twice for 10 s each. After centrifugation at 5,000 rpm for 10 min, absorbance ($A_1$) of the upper layer was measured at 350 nm against isooctane as a blank. The upper layer (5 ml) was then transferred to a 10 ml test tube, and added with *p*-An solution consisted of 0.25 % *p*-An in glacial acetic acid (1 ml). After incubation at room temperature for 10 min, absorbance ($A_2$) was measured at 350 nm against isooctane containing *p*-AnV as a blank. *p*-AnV was determined using the equation (AOCS, 1997):

$$p - AnV = \frac{25 \times (1.2 \times (A_2 - A_1))}{2}$$

### 5.4.6 Rheological property

**- Rheological measurement:** Rheological measurement was performed using a rheometer (Haake RS75, TA Instrument, New Castle, DE, USA) equipped with a stainless steel parallel plate (3.5 cm diameter) with a gap setting of 1 mm. Dynamic oscillatory, steady state flow, and creep and recovery tests were performed. The measurement temperature was controlled at 25 °C using a circulation bath and a controlled Peltier system. One tablespoon of sample was placed at a center of the plate, and excess sample was removed from the edges of the plate. After reach to a steady

state, a power-law model flow test was performed based on a raw data fitting, according to the equation

$$\eta = k\,\gamma^{(n-1)}$$

where $k$ is consistency coefficient, $\gamma$ is the shear rate (s$^{-1}$), $n$ is flow behavior index, and $\eta$ is the viscosity (Pa.s) (Bortnowska *et al.,* 2014).

### 5.4.7 Sensory Evaluation

Sensory analysis was carried out in a sensory laboratory. The dressing (10 g) was placed on a white plastic glass labeled with a three-digit code and served to a panelist with green oak, carrot, and cone (10 g) in a random order. The evaluation was performed by 40 panelists. The attributes and sensory descriptions involving appearance, color, odor, viscosity, taste and overall likeness were evaluated using 9-points hedonic scale, with 1 being dislike extremely and 9 being like extremely (Mihov *et al.,* 2012). Each attribute has its own individual scale.

### 5.4.8 Statistical analysis

All experiments were carried out in triplicate, and the mean values with standard deviations were present. Completely Randomized Design was used. Statistical analysis of the data was performed by analysis of variance (ANOVA) using Duncan's multiple range test (SPSS for Windows, SPSS Inc., Chicago, IL, USA) at a 95 % confidence level.

## 5.5 Results and Discussion

Salad dressings were prepared employing VCO as a dispersed phase, and their characteristic was observed in comparison with the dressings made from SBO. Effect of TMD incorporation at various concentrations (0, 0.5, 1.5, and 3 %) on the physicochemical stability of the dressings was also elucidated.

To observe colloidal stability of the dressings, the initial oil droplet diameter of the dressings was measured, and the result was shown in **Figure 10**.
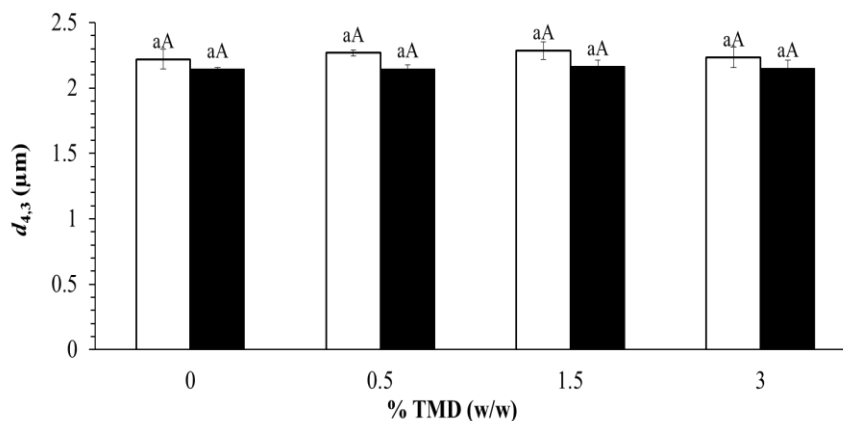
**Figure 10** Initial mean diameters ($d_{4,3}$) of the salad dressings made from VCO (open bar) and SBO (closed bar) at various TMD concentrations (0, 0.5, 1.5 and 3%). Mean value ± standard deviation (n=3) were shown.

Different lower letters indicate significant difference between means in a same type of oil (P<0.05).

Different upper letters indicate significant difference between means in a same TMD concentration (P<0.05).

The initial oil droplet diameters of all salad dressings were in the range of 2.14–2.28 µm. There was no significant difference on initial $d_{4,3}$ of the dressings containing TMD at dissimilar contents (P>0.05). This behavior was in accordance with the study of Klinkesorn *et al.* (2004) reporting no difference on mean droplet diameter of corn oil emulsions containing maltodextrin at different concentrations (0–35 wt %). Different initial $d_{4,3}$ of the dressings prepared using different oils was not noticeable (P>0.05).

Next, long term stability of the dressing sample was evaluated. **Figure 11** shows a storage time dependence on oil droplet size of the dressing samples. By incorporating TMD, droplet size increasing could be prohibited through 3 weeks of storage, irrespectively of oil type. For both VCO and SBO dressings, using TMD at the concentrations of 1.5 and 3 % could lower rate of drop size increasing through 8 weeks of storage (P<0.05). Salad dressings are thermodynamically unstable system, in which aggregation of oil droplets tended to be observed with a prolonged storage time (Waite *et al.,* 2009). TMD might function as a thickening agent to increase viscosity of the dressing, thereby delaying drop aggregation (Claesson *et al.,* 2003). Different

increasing in $d_{4,3}$ with storage time between SBO and VCO dressings was not noticeable (P>0.05).
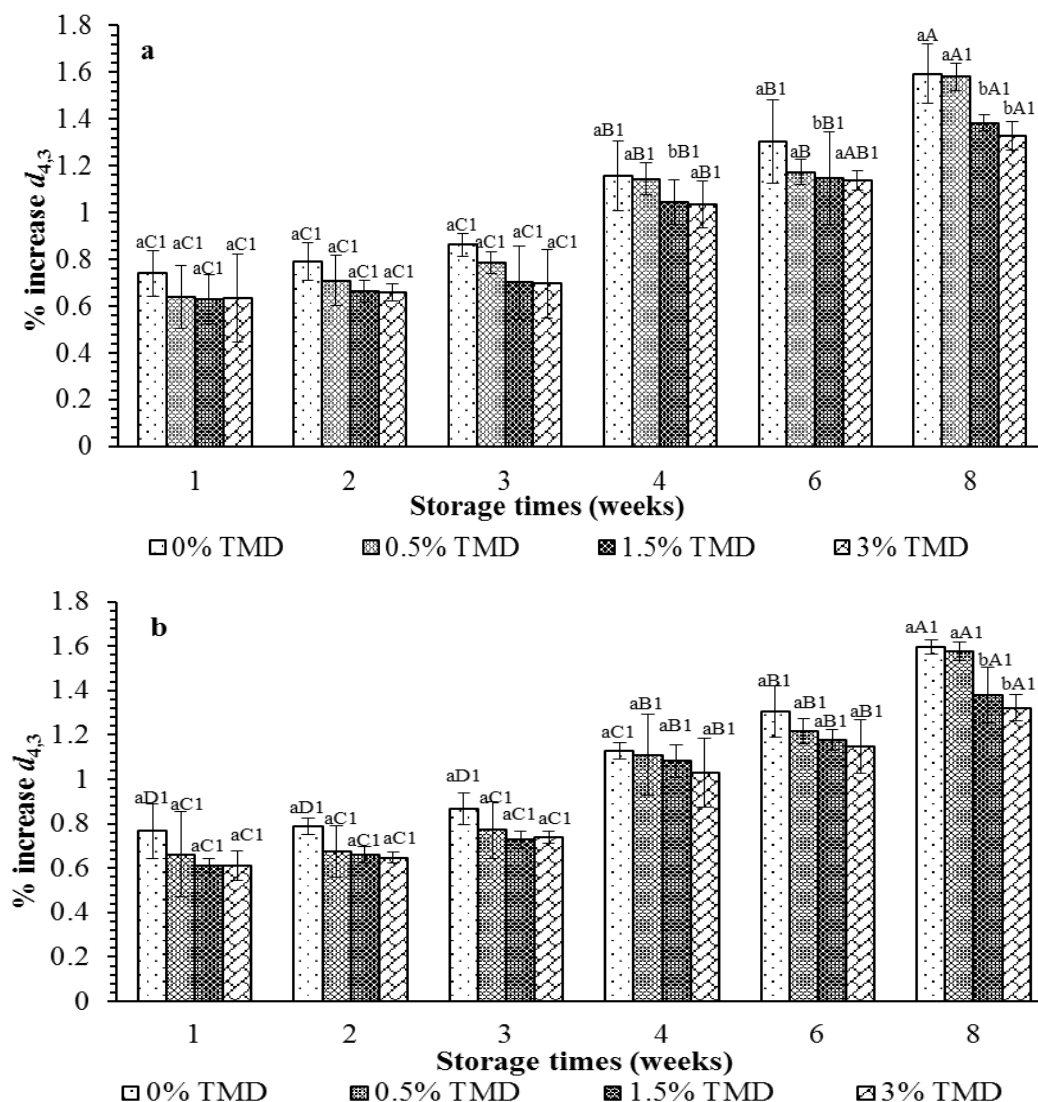


**Figure 11** Percentage of $d_{4,3}$ increasing of the salad dressings made from VCO (a) and SBO (b) at different TMD concentrations and storage times (0–8 weeks). Mean value ± standard deviation (n=3) were shown.

In each subfigure, different lower letters indicate significant difference between means in a same storage times (P<0.05).

In each subfigure, different upper letters indicate significant difference between means in a same TMD concentration (P<0.05).

Different numeric letters indicate significant difference between means at a same TMD concentration and storage time (P<0.05).

Further, CI of the dressings was observed as a function of storage times, and the result was shown in **Figure 12**. By adding TMD, CI of the dressing samples could be successfully lowered, especially at the increased TMD concentration. The lowest CI was observed for the dressings added with 3 % TMD for both VCO and SBO dressings (P<0.05). This behavior was in a good agreement with the report of Parker *et al.* (1995) and Udomrati *et al.* (2011) suggesting that polysaccharides could form a three dimensional gel network in emulsified matrix that could retard flocculation of oil droplets, thereby delaying creaming process. For the control sample, CI increased thoroughly with increased storage time (P<0.05), suggesting to an inferior dispersibility of the samples. The emulsions incorporated with TMD at 3 % were stable over a week period as implied by no noticeable CI increase (P>0.05). For the model emulsions containing TMD with DE of 9 at different concentrations (0–40 %), increasing TMD concentration could reduce creaming rate of the system during storage (Udomrati *et al.,* 2011). CI indirectly indicated degree of drop flocculation since a creaming rate increases with a progressive of flocculation process (Sun *et al.,* 2007). It was noted that a tendency of CI increasing with prolonged storage time was in accordance with increased degree of drop aggregation (see **Figure 11**).

Change in color of the dressings was observed during a storage period of 8 weeks, and the results were shown in **Figure 13**. The $\Delta E$ value of all samples was significantly increased after 8 weeks of storage (P<0.05). This behavior was in agreement with the report of Ma *et al.* (2013). After 8 weeks, the highest $\Delta E$ was found for the control dressing (P<0.05), irrespectively of oil types. By incorporating TMD, the $\Delta E$ of dressings could be decreased, suggesting to the ability of TMD to retain color appearance of the samples. It should be noted that lowered change in color was also observed in mayonnaise and yogurt with a good stability (Tseng and Zhao, 2013). Lowered $\Delta E$ of TMD added dressings was more likely due to lowered change in droplet size of the emulsions as suggested by the previous result (see **Figure 11**). With different size, emulsified droplets might reflect light in different manners, thereby affecting to color of the products (Laca *et al.,* 2010; Tseng and Zhao, 2013). From the present result, $\Delta E$ was remarkably affected by oil type. The VCO dressings possessed lower $\Delta E$ than those of SBO counterparts, regardless of TMD concentration and storage time. This

result indicated that the color of dressings could be maintained effectively by employing VCO as a dispersed phase.
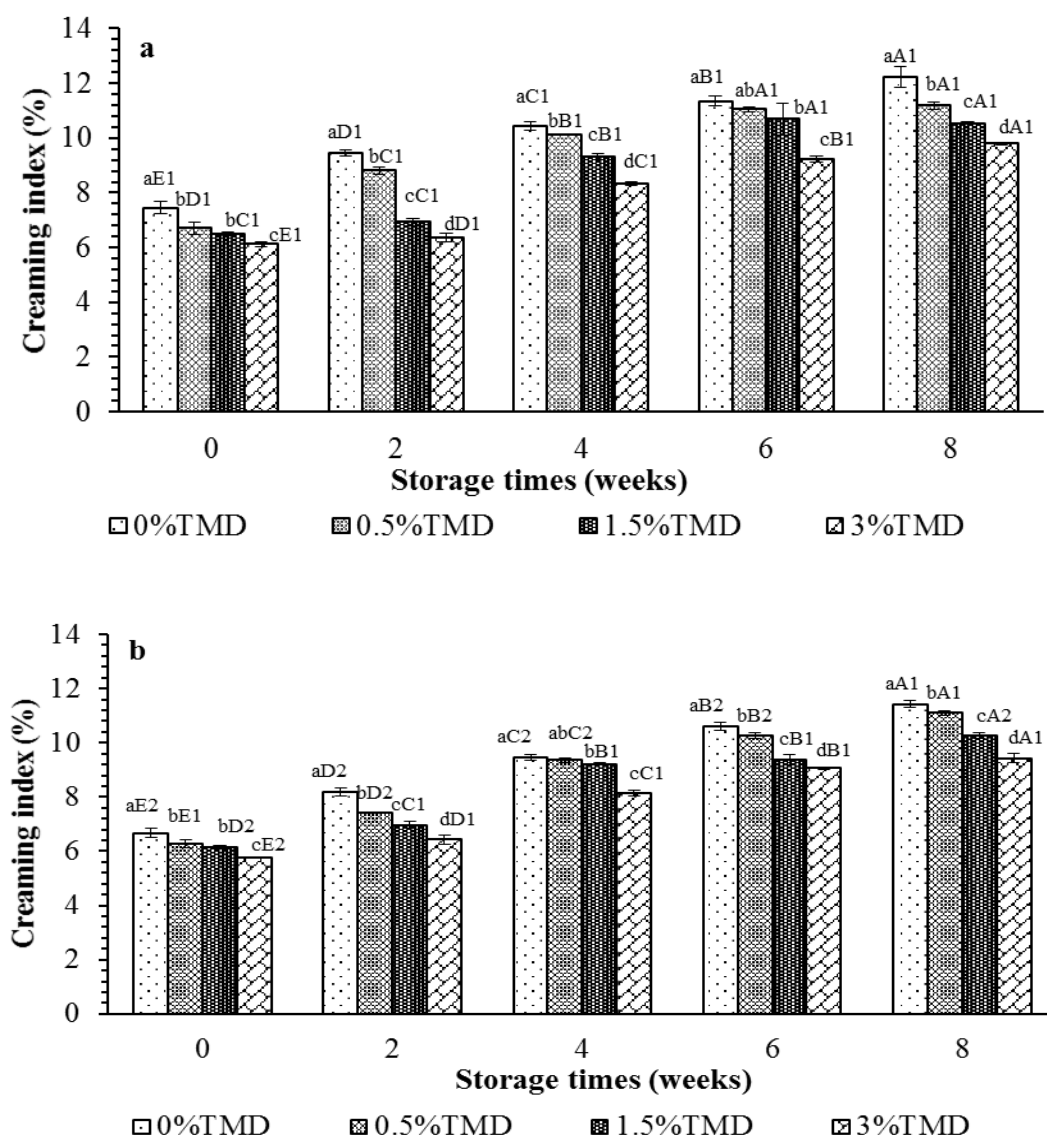


**Figure 12** Creaming index (CI) of the salad dressings made from VCO (a) and SBO
(b) at different TMD concentrations and storage times (0–8 week). Mean
value ± standard deviation (n=3) were shown.

In each subfigure, different lower letters indicate significant difference between means in a same storage
times (P<0.05).

In each subfigure, different upper letters indicate significant difference between means in a same TMD
concentration (P<0.05).

Different numeric letters indicate significant difference between means at a same TMD concentration
and storage time (P<0.05).

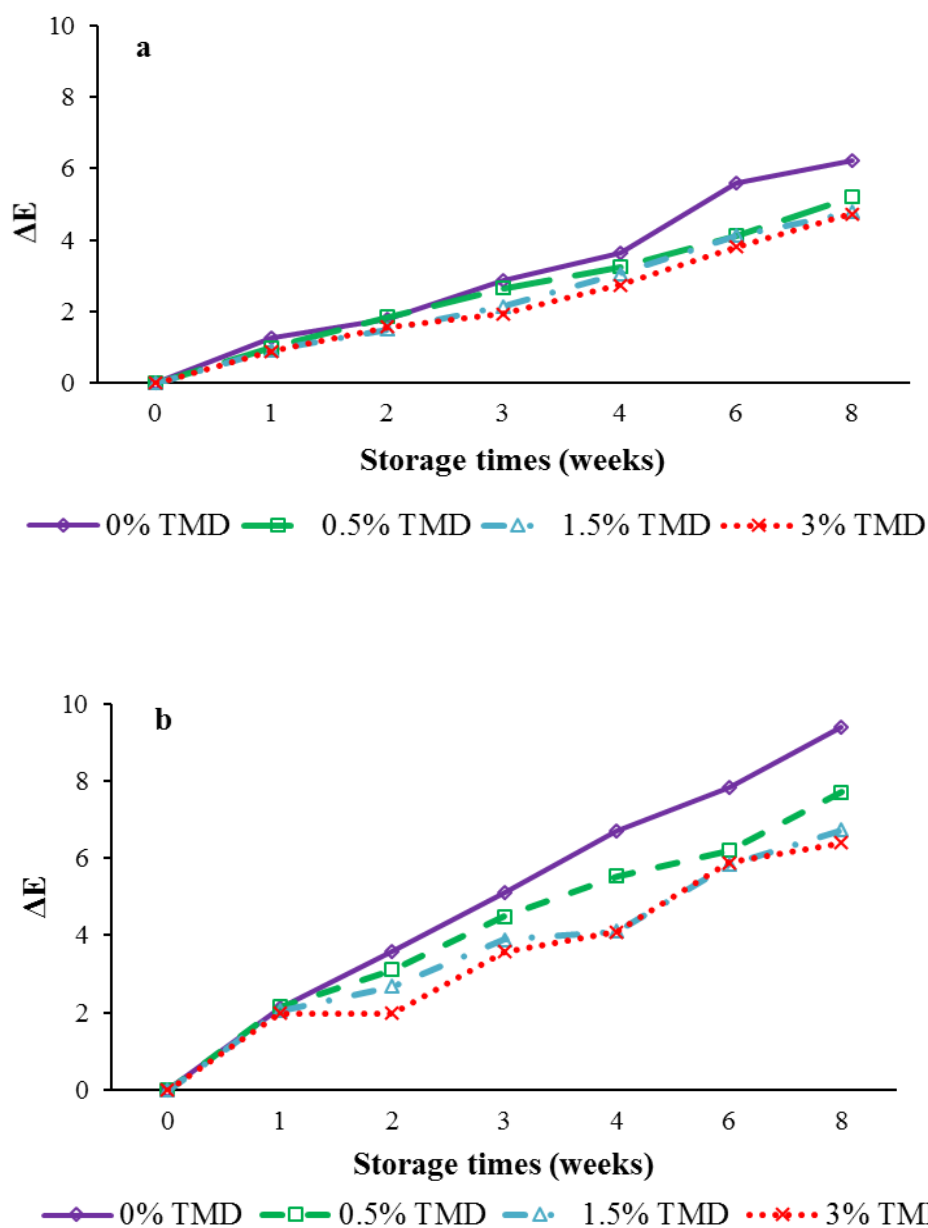**Figure 13** Storage time dependence on $\Delta E$ value of the salad dressings made from VCO (a) and SBO (b) containing TMD at different concentrations (0, 0.5, 1.5, and 3%) at various storage times (0–8 week). Mean value ± standard deviation (n=3) were shown.

Next, pH change of the dressings at various storage times was measured as illustrated in **Figure 14**. Within 4 weeks of storage, pH of all dressings was retained in a range of 3.55–3.61. Afterward, decreasing of pH with storage time was observed through 8 weeks for all dressing samples, especially for the control ($P>0.05$). This tendency was in agreement with the previous works showing the diminishing of pH of Italian and French dressings with increased storage time (Waite *et al.,* 2009; Tseng and Zhao, 2013). Reduction of pH with prolonged storage time could be expected due to a production of lactic acid and galactose caused by bacterial metabolic activity (Tseng and Zhao, 2013). Moreover, lipid oxidation could also affect to decrease pH of emulsion system (McClements and Decker, 2000). Free fatty acids emerged by hydrolysis of triglycerides could be also accounted for a pH lowering in emulsion system (Herma and Grove, 1993; McClements and Decker, 2000). Incorporation of TMD could retard pH lowering of the dressings. This behavior implied that stability of the dressings could be improved by incorporating TMD.

Further, rheological property of the dressing samples was investigated. Rheological characteristic of emulsions had a significant role on stability and engineering calculations for handling, designing, evaluating, and operating food processing equipment, such as mixing and piping equipment and pump (Maskan and Gogus, 2000). In this study, a power-law mathematical model was performed to determine rheological behavior of the dressings, because it is widely used to describe flow behavior of emulsified food products (Izidoro *et al.,* 2009). **Table 16** and **Figure 15–16** show rheological behavior of the dressing samples at various storage times.
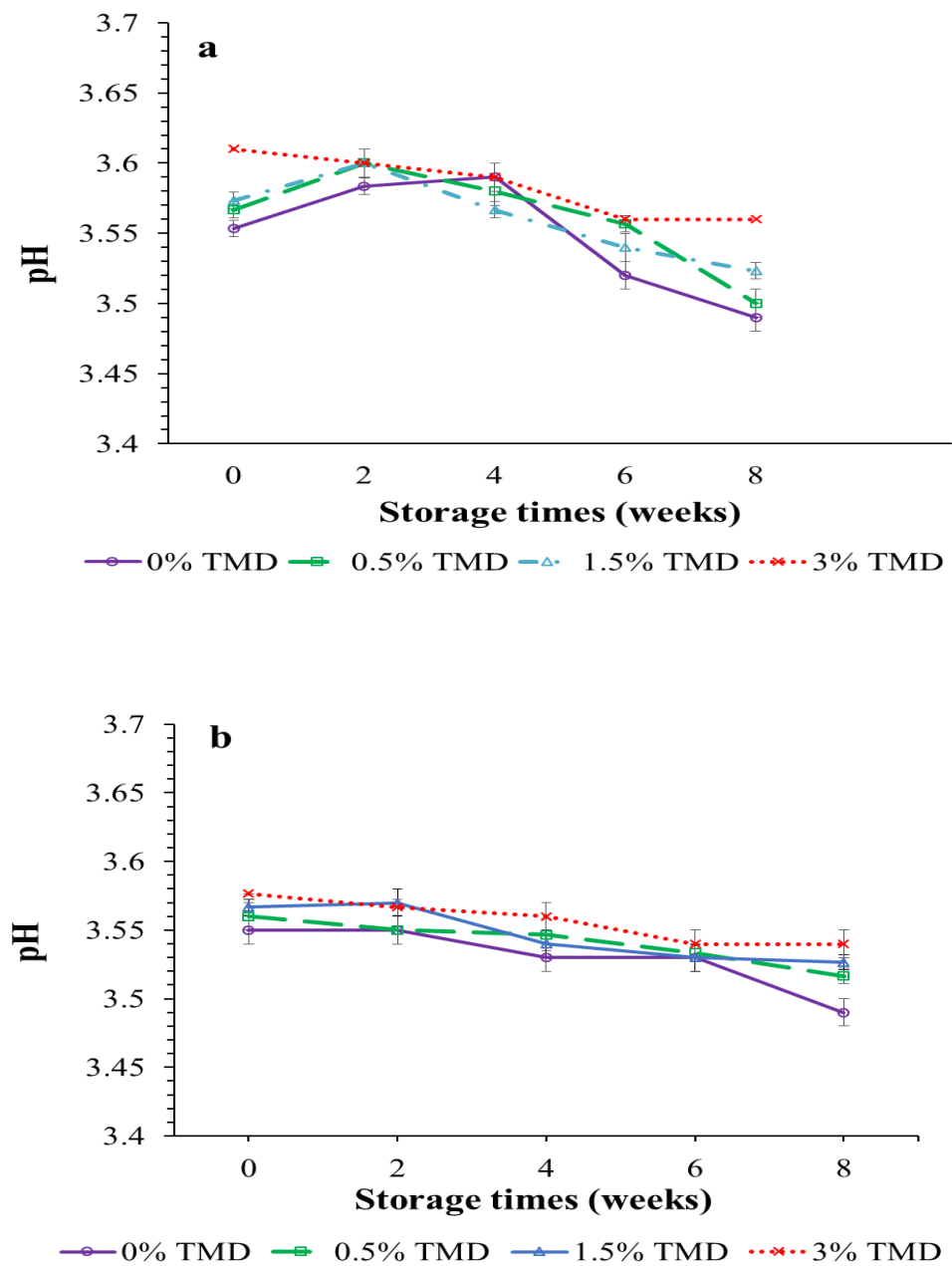
**Figure 14** Storage time dependence on pH of the VCO (a) and SBO (b) dressings added with TMD at various concentrations. Mean value ± standard deviation (n=3) were shown.

Over the observed shear rate range of 0.01 to 300 s^{-1}, all dressing samples possessed flow behavior index (*n*) within the range of 0.4–0.6, suggesting to a shear-thinning (pseudoplastic) behavior (Zhen and Joyce, 2013). The *n* values of dressing samples were within the range of 0.4–0.6, implying to Non-Newtonian flow behavior.

Normally, the fluid with non-Newtonian flow behavior showed a decreasing of viscosity with increased shear stress, which was in agreement with the result of present work as shown in **Figure 15** and **Figure 16**. Increase TMD concentration thoroughly affected to increase viscosity (P<0.05) of the samples, as evidenced by increasing of consistency coefficient ($k$) (**Table 16**). In the range of observed shear rates, the viscosity of all salad dressings added with TMD was generally higher than the control, and the highest viscosity was observed when TMD was added at 3%. This behavior suggested that TMD had a significant role to increase viscosity of the system. This was more likely due to TMD could act as a thickening agent (Paredes *et al.,* 1989), attributed to ability of long-chain glucose unit fractions to increase flow resistance (Ibanoglu, 2002). Other studies also reported increased emulsion viscosity in a presence of hydrocolloid, especially at increased concentration (Mandala *et al.,* 2004; Zhen and Joyce, 2013). Increased viscosity of the dressings containing TMD at higher concentration was coincident with the lowered % increase $d_{4,3}$ (**Figure 11**) and CI (**Figure 12**) of the dressings. Increasing viscosity might affect to immobilize dispersed oil droplets in a weak gel-like network (Dickinson, 2003), thereby retarding drop aggregation. Considering on the effect of storage time, all of the dressings generally showed a decreasing in viscosity with a prolonged storage time. This might be postulated due to increasing of oil droplet size as suggested by the result in **Figure 11**. As a result of size increasing, the emulsified drops were less structured, so less interaction between neighboring dispersed drops could be expected, leading to decrease viscosity of the system (Zhen and Joyce, 2013). Considering on the effect of oil type, SBO dressings showed significantly higher $k$ value than the VCO dressings (P<0.05), which might be expected since a higher viscosity of SBO than VCO. The viscosities of soybean and coconut oil were reported as 31.8 and 28 cP, respectively (Nourredini *et al.,* 1992). The present result indicated that viscosity played a dominant role in creaming rate of the emulsions, in which the creaming stability improved with increase viscosity. Nonetheless, there was no significantly different colloidal stability between SBO and VCO dressings in the present study, as suggested by comparable % increase $d_{4,3}$ (see **Figure 11**).

**Table 16** Consistency coefficient ($k$) and flow behavior index ($n$) values of the salad dressings made from VCO and SBO at different TMD concentrations (0–3 %) and storage times (0–8 weeks)

| Storage times (week) | | VCO | | | | SBO | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0% | 0.5% | 1.5% | 3% | 0% | 0.5% | 1.5% | 3% |
| W0 | $k$ | 12.48±0.61[dA2] | 14.72±1.41[cA2] | 17.8±0.16[bA2] | 18.97±0.27[aA2] | 16.13±0.92[dA1] | 18.42±0.1[cA1] | 22.12±1.28[bA1] | 23.55±0.29[aA1] |
| | $n$ | 0.47±0.02[aD1] | 0.48±0.02[aE1] | 0.45±0.01[bE1] | 0.44±0.01[cD1] | 0.44±0.01[aF1] | 0.47±0.01[bE1] | 0.43±0.01[cF2] | 0.4±0.01[dF2] |
| W1 | $k$ | 11.48±0.61[cA2] | 14.72±1.41[bA2] | 17.8±0.16[aA2] | 18.97±0.27[aB2] | 16.13±0.93[cB1] | 18.42±0.1[bB1] | 22.12±1.28[aA1] | 23.55±0.29[aB1] |
| | $n$ | 0.47±0.02[aD1] | 0.48±0.02[aE1] | 0.45±0.01[bDE1] | 0.44±0.01[bC1] | 0.47±0.01[aE1] | 0.43±0.01[bF2] | 0.43±0.01[bE2] | 0.4±0.01[cF2] |
| W2 | $k$ | 11.04±0.93[dAB2] | 12.72±0.32[cB2] | 15.77±0.15[bB2] | 16.98±0.4[aC2] | 14.41±0.24[cC1] | 16.76±0.22[bC1] | 17.3±0.45[bB1] | 20.86±0.46[aC1] |
| | $n$ | 0.5±0.02[aCD1] | 0.51±0.02[aD1] | 0.46±0.02[bCD1] | 0.45±0.01[bC1] | 0.47±0.01[aE2] | 0.44±0.01[bE2] | 0.43±0.01[cE2] | 0.44±0.01[cE2] |
| W3 | $k$ | 9.07±0.97[dCD2] | 11.2±0.13[cC2] | 14.11±0.8[bC1] | 16.58±0.08[aC1] | 12.33±0.11[dD1] | 13.79±0.44[cD1] | 14.97±0.98[bC1] | 17.39±0.5[aD1] |
| | $n$ | 0.52±0.02[aC1] | 0.52±0.01[aABC1] | 0.47±0.01[bC1] | 0.45±0.01[bC1] | 0.51±0.01[aD1] | 0.46±0.01[bD2] | 0.45±0.01[cD1] | 0.45±0.01[cD1] |
| W4 | $k$ | 9.99±1.35[bBC1] | 10.07±0.87[bCD2] | 13.18±0.97[aC1] | 14.65±0.43[aD1] | 11.1±0.73[dE1] | 12.74±0.4[cE1] | 13.82±0.43[bC1] | 15.32±0.02[aE1] |
| | $n$ | 0.49±0.01[bCD1] | 0.52±0.01[aABC1] | 0.47±0.01[cC1] | 0.49±0.01[bcB1] | 0.52±0.01[aC1] | 0.51±0.01[bC1] | 0.46±0.01[cC1] | 0.46±0.01[cC2] |
| W6 | $k$ | 7.65±0.55[cDE2] | 8.96±0.47[bDE2] | 12.01±0.9[aD1] | 12.88±0.43[aE1] | 9.94±0.92[cF1] | 10.15±0.16[cF1] | 12.33±0.38[bD1] | 13.74±0.25[aF1] |
| | $n$ | 0.56±0.02[aB1] | 0.54±0.02[aB1] | 0.5±0.01[bB1] | 0.51±0.01[bA1] | 0.54±0.01[aB2] | 0.52±0.01[aB2] | 0.5±0.01[bB1] | 0.5±0.01[bB1] |
| W8 | $k$ | 7.39±0.31[dE2] | 8.22±0.32[cE2] | 10.01±0.18[bE2] | 11.5±0.45[aF1] | 8.63±0.26[dG1] | 9.44±0.19[cG1] | 11.74±0.53[bD1] | 12.5±0.19[aG1] |
| | $n$ | 0.61±0.02[aA1] | 0.58±0.01[bA1] | 0.52±0.01[cA1] | 0.51±0.01[cA1] | 0.57±0.01[aA2] | 0.54±0.01[bA2] | 0.52±0.01[cA1] | 0.52±0.01[cA1] |

Mean value ± standard deviation ($n=3$) were shown.

In a same oil type, different lower letters indicate significant difference between means in a same row (P<0.05).

In a same oil type, different upper letters indicate significant difference between means in a same column (P<0.05).

Different numeric letters in a same oil indicate significant difference between means at a same concentration of TMD and storage time (P<0.05).
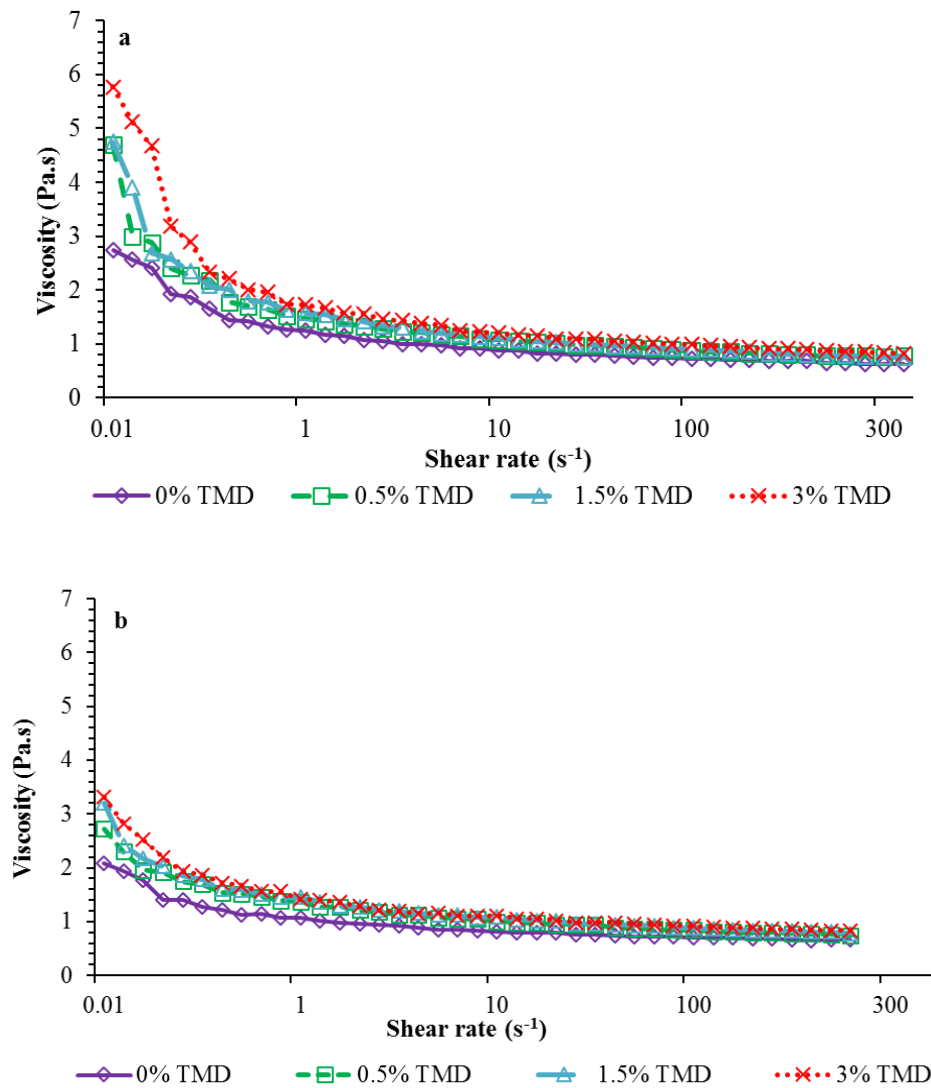
**Figure 15** Viscosity versus shear rate of the VCO dressings after storage for 0 week (a)
and 8 weeks (b). The dressings contained TMD at various concentrations (0,
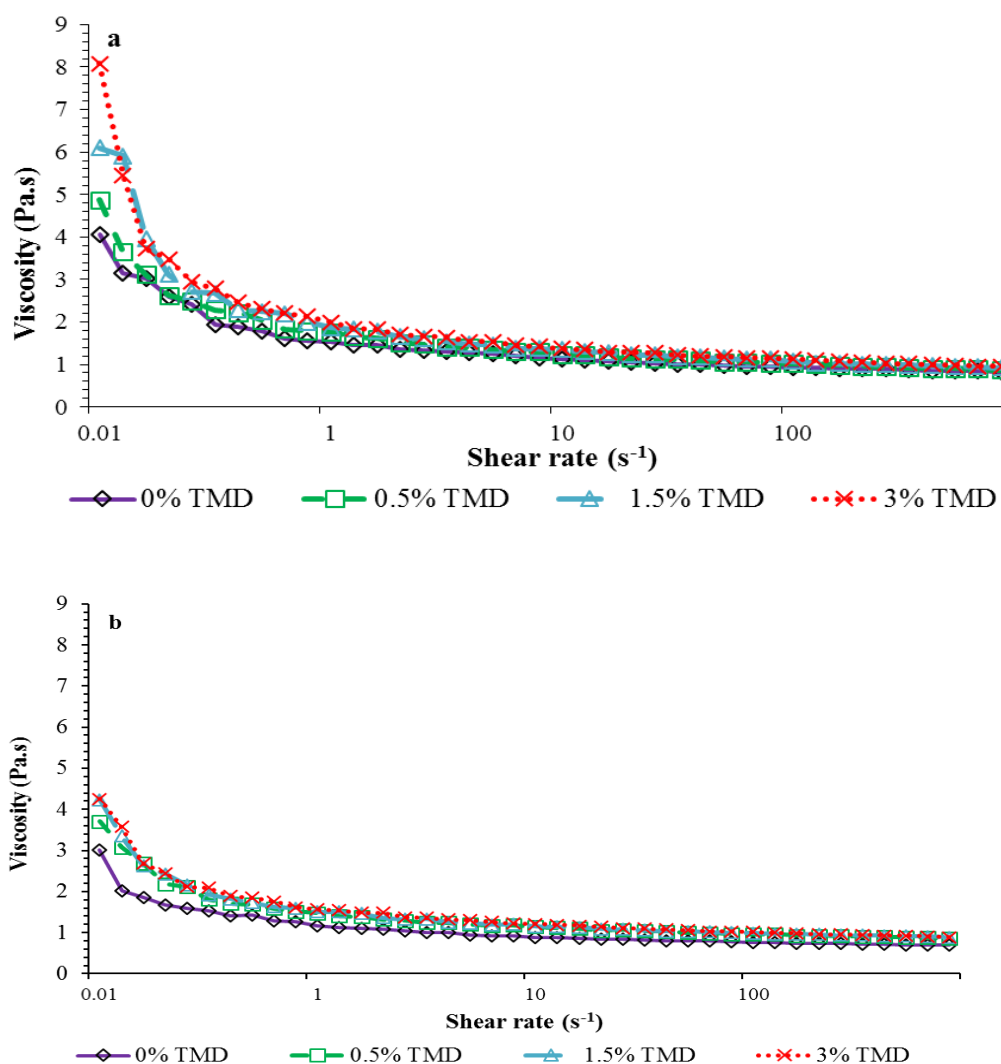0.5, 1.5 and 3 %).

**Figure 16** Viscosity versus shear rate of the SBO dressings after storage for 0 week (a) and 8 weeks (b). The dressings contained TMD at various concentrations (0, 0.5, 1.5 and 3 %).

By employing different types of oil, physicochemical characteristics of the emulsion might be affected. Phenolic compounds possessed an important role to enhance oxidative stability of food product. Nonetheless, other factors, such as moisture content, pH, temperature, as well as a presence of oxygen, light, metal ions and enzymes could remarkably influence retention of polyphenols, thereby affecting to oxidative stability of the system (Mazza, 1995). To more elucidate characteristics of the dressing, total phenolic content (TPC) present in the dressing samples was monitored as a function of storage times, and the result was illustrated in **Figure 17**.
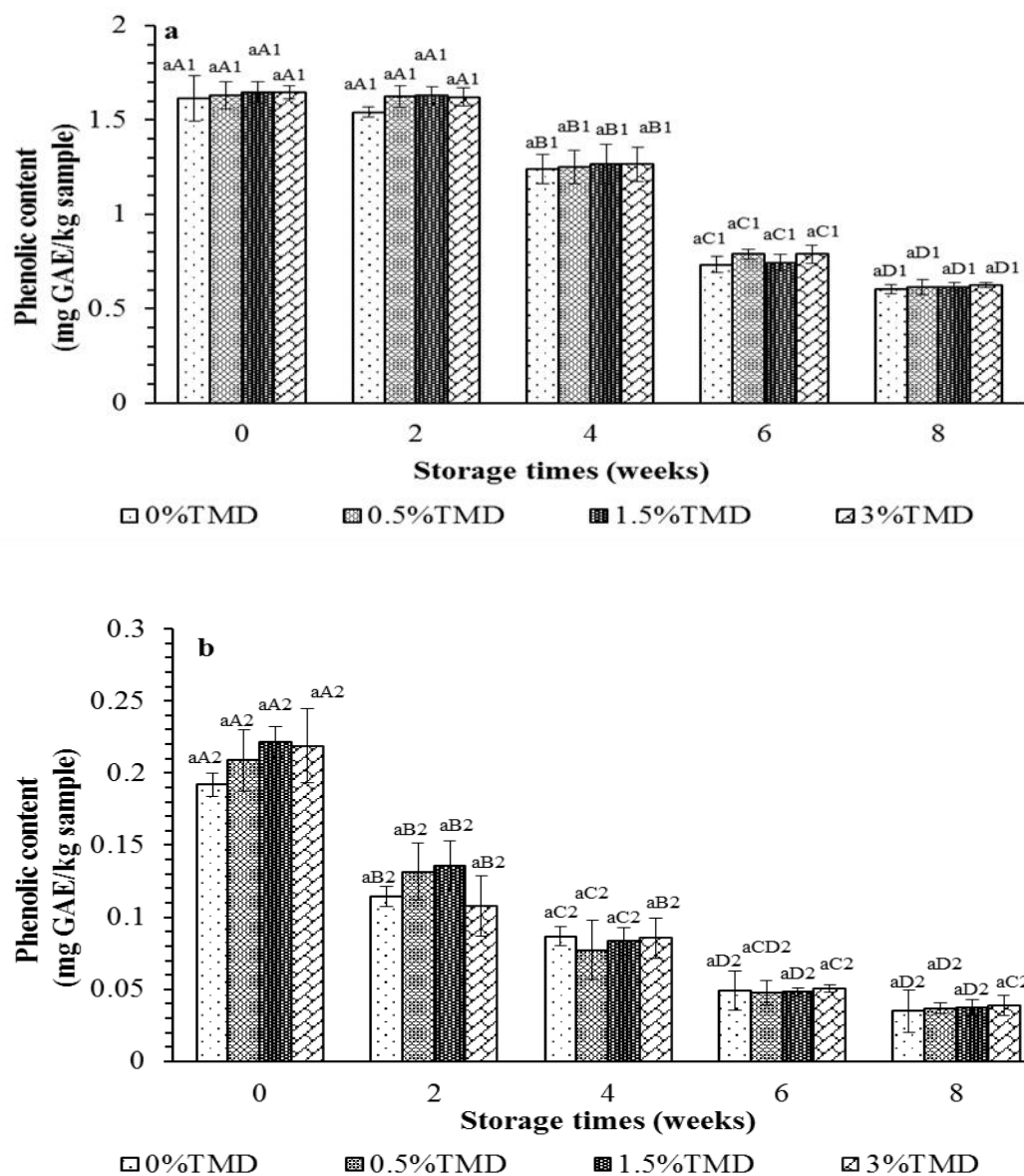
**Figure 17** Total phenolic content (TPC) (mg GAE /kg sample) of the salad dressing made from VCO (a) and SBO (b) containing TMD at different concentrations (0–3%) at various storage times (0–8 week). Mean value ± standard deviation (n=3) were shown.

In each subfigure, different lower letters indicate significant difference between means in a same storage times ($P<0.05$).

In each subfigure, different upper letters indicate significant difference between means in a same TMD concentration ($P<0.05$).

Different numeric letters in a same oil indicate significant difference between means at a same concentration of TMD and storage time ($P<0.05$).

From **Figure 17**, the dressings made from VCO contained obviously higher TPC compared to the SBO counterparts (P<0.05). This could be attributed to a minimal heating process involved in VCO production compared to SBO (Henna and Tan, 2009). TPC of the vegetable oils could be affected by heat exposed and refining process. Generally, unrefined oils possessed higher TPC than refined vegetable oils, because some contents of natural polyphenols could be degraded through refining process (Garcia *et al.,* 2006). TPC of the dressing samples tended to decrease with increase storage time. For the VCO dressings, TPC reduced significantly after 4 weeks of storage (P<0.05), whereas SBO dressings showed a markedly reduced TPC since 2 weeks (P<0.05). This suggested more pronounced degradation of phenolic compounds of the SBO dressings compared to VCO counterparts, which might be related to better oxidative stability of the later samples (Henna and Tan, 2009). From the present result, TMD concentration had no significant effect on TPC of the dressing samples (P>0.05).

Lipid oxidation is one of the major concerns in food quality deterioration by generating off-flavor compounds. Oxidative process can be accelerated by light, heat, enzymes, metals, metalloproteins and microorganisms (Shahidi and Zhong, 2005). To monitor degree of lipid oxidation, development of PV, TBA, and *p*-AnV of the dressings during storage of 8 weeks was shown in **Figure 18, 19** and **20**, respectively.

**Figure 18** PV of the salad dressings made from VCO (a) and SBO (b) containing TMD
at different concentrations at various storage times. Mean value ± standard
deviation (n=3) were shown.

In each subfigure, different lower letters indicate significant difference between means in a same storage
times (P<0.05).

In each subfigure, different upper letters indicate significant difference between means in a same TMD
concentration (P<0.05).

At a same TMD concentration and storage time, the PV of VCO and SBO dressings was thoroughly
significant difference (P<0.05).
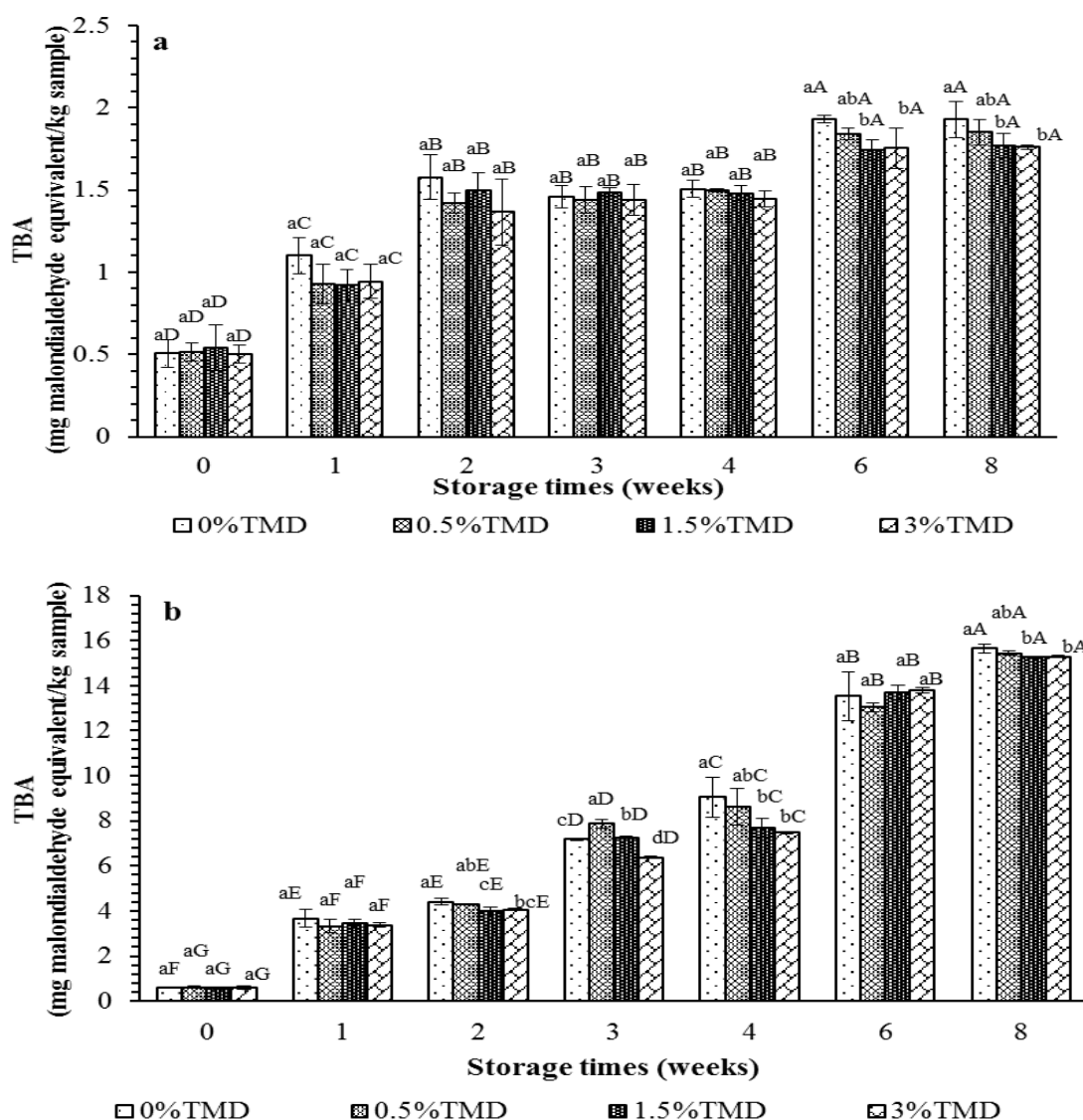
**Figure 19** TBA of the salad dressings made from VCO (a) and SBO (b) containing TMD at different concentrations at various storage times. Mean value ± standard deviation (n=3) were shown. In each subfigure, different lower letters indicate significant difference between means in a same storage times (P<0.05).

In each subfigure, different upper letters indicate significant difference between means in a same TMD concentration (P<0.05).

In each test parameter, different numeric letters in a same oil indicate significant difference between means at a same concentration of TMD and storage time (P<0.05).

At a same TMD concentration and storage time, the PV of VCO and SBO dressings was thoroughly significant difference (P<0.05).
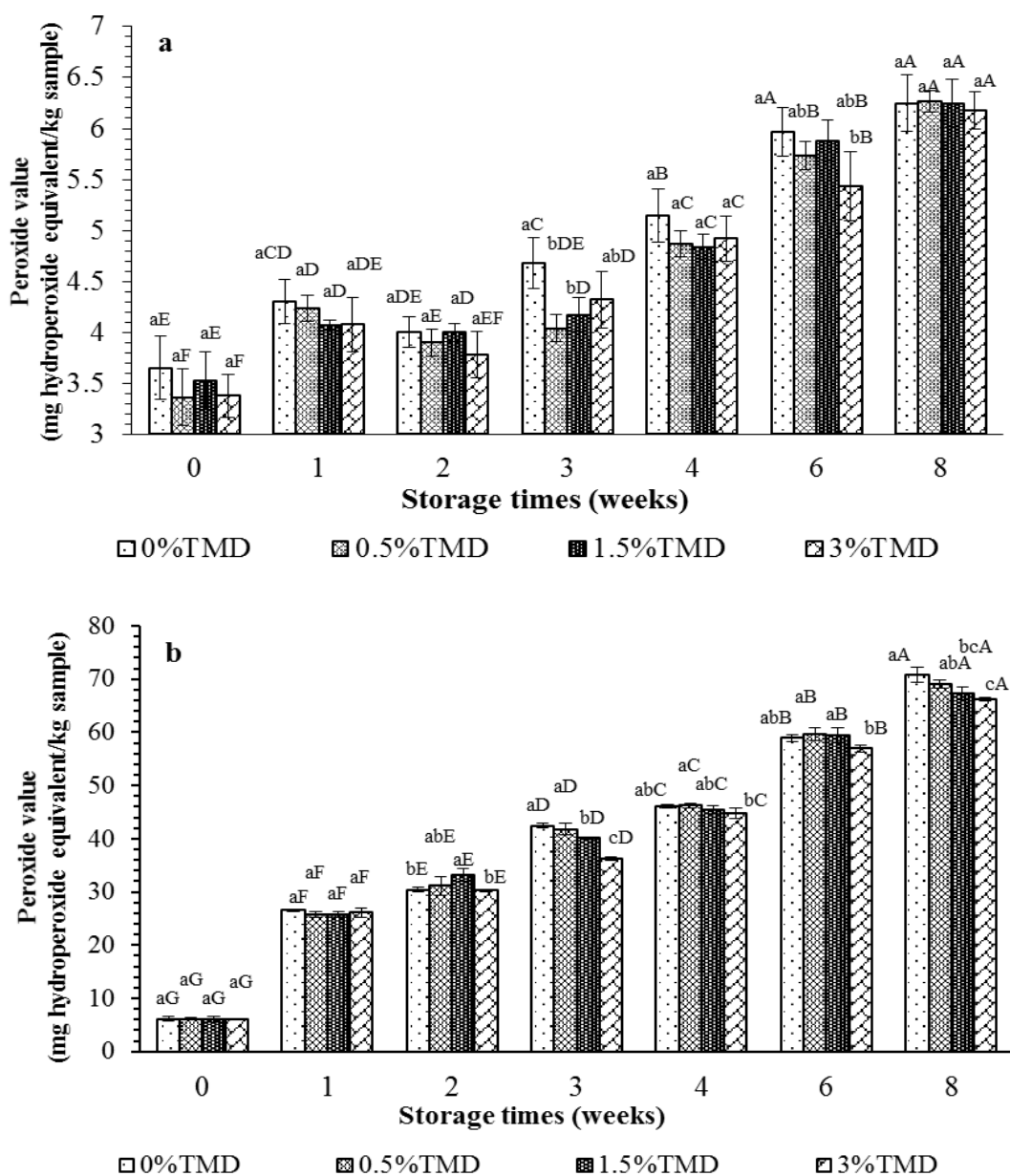
**Figure 20** *p*-AnV of the salad dressings made from VCO (a) and SBO (b) containing TMD at different concentrations at various storage times. Mean value ± standard deviation (n=3) were shown.

In each subfigure, different upper letters indicate significant difference between means in a same TMD concentration (P<0.05).

In each test parameter, different numeric letters in a same oil indicate significant difference between means at a same concentration of TMD and storage time (P<0.05).

At a same TMD concentration and storage time, the PV of VCO and SBO dressings was thoroughly significant difference (P<0.05).

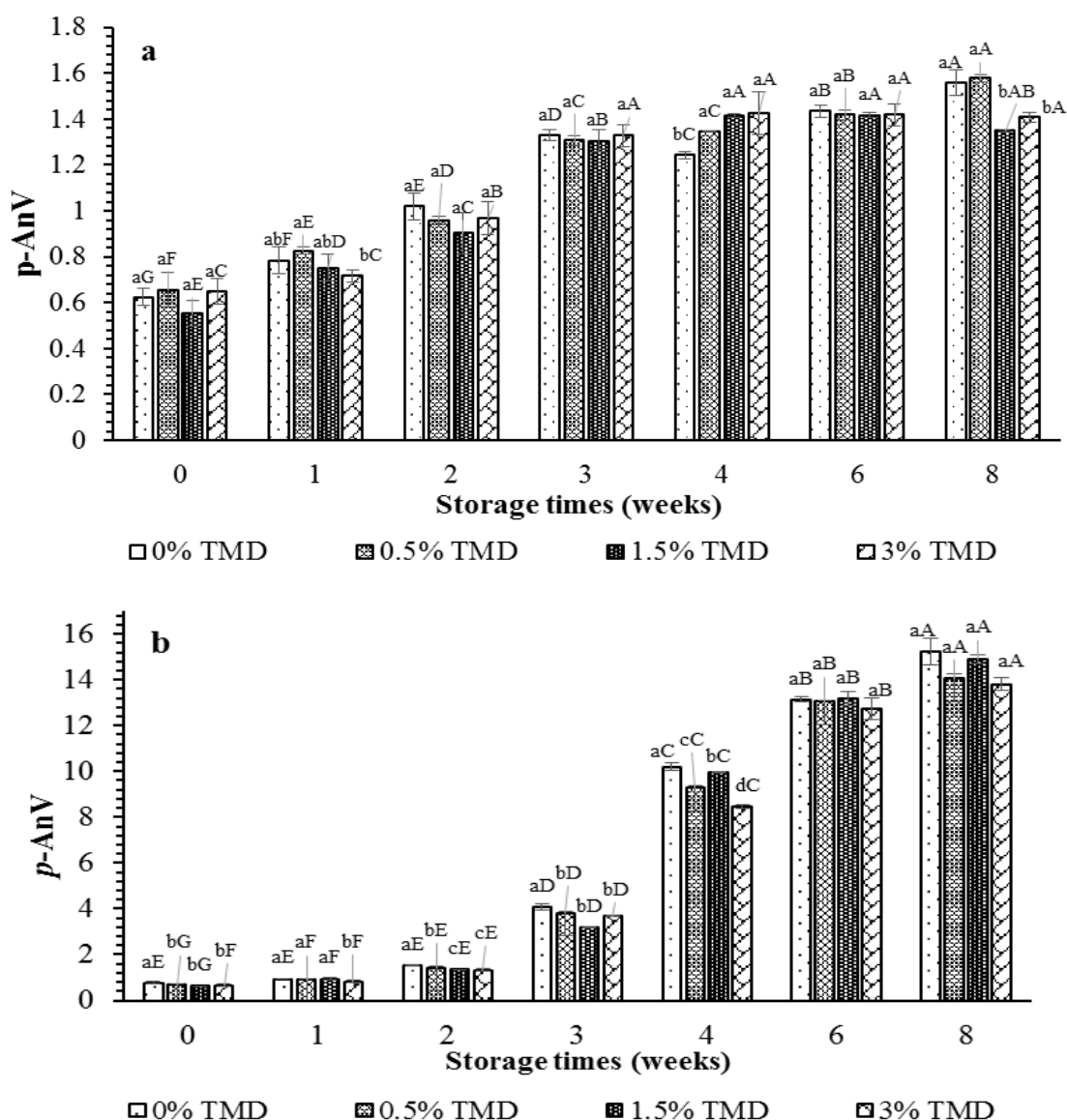To evaluate degree of lipid oxidation, PV was monitored to quantify primary products (hydroperoxide), whereas TBA and *p*-AnV were related to secondary products

(malondialdehyde and 2-alkenals, respectively) of the reaction. Degree of lipid oxidation was increased thoroughly with a prolonged storage time (P<0.05). PV, TBA and *p*-AnV of the salad dressings made from VCO increased from 3.36–3.65 mg hydroperoxide equivalent/kg sample, 0.5–0.54 mg malondialdehyde equivalent/kg sample, and 0.55–0.65 in the first week to 6.18–6.26 mg hydroperoxide equivalent/kg sample, 0.58–0.6 mg malondialdehyde equivalent/kg sample, and 0.71–0.82 after 8 weeks of storage, respectively. Emulsion was prone to lipid oxidation, because of a high ratio of surface area/oil volume, resulting in a greater exposure area of oil phase to water soluble pro-oxidants (McClements and Decker, 2000). Generally, TMD concentration had no significant effect on PV, TBA and *p*-AnV of the dressings (P<0.05). Nonetheless, the lowered TBA and *p*-AnV could be observed for the VCO dressings after 6 weeks of storage, when TMD was added at 1.5 and 3 % (P<0.05). Considering on the effect of oil type, higher oxidative degree was clearly found for the dressings made from SBO compared to the VCO counterparts (P<0.05). Greater susceptibility of SBO emulsions against lipid oxidation could be expected since a higher unsaturation degree of composited fatty acids and lower TPC of SBO compared to VCO. Phenolics could be potently employed as a natural antioxidant in various food products (Toyosaki, 1992). Phenolic compounds possessed antioxidative ability through various mechanisms such as chain-breaking by donating hydrogen to peroxyl, alkoxyl and lipid alkyl radicals and converting them to non-radical products (Reische, 2008). The resulting antioxidant radical was stabilized by delocalization of unpaired electrons which made it relatively nonreactive and unlikely to promote further oxidation (Frankel, 1993). Frankel *et al.* (1994) and Huang *et al.* (1996) showed that the relative antioxidant effectiveness of phenolics depends on the lipid substrate, physical state, concentration, oxidation time and temperature, as well as analytical method used to determine oxidation degree. It should be noted that better oxidative stability of VCO dressings than the SBO counterparts was coincidental with less color change (**Figure 13**) and lower pH diminish (**Figure 14**) at different storage times of the former samples.

From all point of views, using 3 % TMD could successfully improve stability of dressing products as indicated by lower droplet size increasing and CI as well as less change in $\Delta E$ and pH with storage time. Therefore, sensory evaluation of the VCO and

SBO dressing samples was performed at this TMD level. **Table 17** shows the sensory scores of the VCO and SBO dressings as affected by TMD adding at 3 %.

**Table 17** Scores of sensory evaluation for salad dressings made from VCO and SBO without and with 3 % TMD

| Attributes | VCO | | SBO | |
|---|---|---|---|---|
| | 0 % TMD | 3 % TMD | 0 % TMD | 3 % TMD |
| **Appearance** | $6.45\pm1.18^b$ | $6.48\pm1.06^b$ | $7.55\pm0.90^a$ | $7.03\pm1.05^a$ |
| **Color** | $6.38\pm1.25^b$ | $6.33\pm1.25^b$ | $7.20\pm1.09^a$ | $7.13\pm0.85^a$ |
| **Odor** | $6.08\pm1.40^a$ | $6.10\pm1.41^a$ | $6.58\pm1.24^a$ | $6.33\pm1.42^a$ |
| **Viscosity** | $6.34\pm0.74^c$ | $7.08\pm0.97^{ab}$ | $6.83\pm1.22^b$ | $7.20\pm0.65^a$ |
| **Taste** | $6.20\pm1.18^b$ | $6.83\pm1.22^a$ | $6.90\pm1.26^a$ | $7.00\pm1.26^a$ |
| **Overall likeness** | $6.33\pm1.23^c$ | $6.93\pm0.80^{ab}$ | $6.68\pm1.16^{bc}$ | $7.18\pm1.01^a$ |

Mean values with standard deviation (n=40) were shown. Different letters indicate significant difference between means in a same row (P<0.05).

SBO dressings possessed higher scores on appearance and color than the VCO dressing (P<0.05). By incorporating TMD, score on taste of the VCO dressings was higher than the recipe without TMD (P<0.05). There was no significant difference on odor score for all dressings (P>0.05), which might be due to a presence of various components such as mustard and vinegar that masked the odor of VCO. Higher acceptance on viscosity was found for the dressings added with TMD, irrespectively of oil type (P<0.05). This result suggested that TMD could modify viscosity of the dressings to preferable range of the panelists. For the VCO dressings, TMD incorporation affected to increase acceptability in taste of the product (P<0.05). Considering on the overall likeness, the highest score was found for the dressings incorporated with 3 % TMD, regardless of oil types. Thus, VCO could be employed to prepare salad dressing with appreciable sensory characteristic, especially in a presence of TMD.

**5.6 Conclusions**

Physicochemical characteristics of the salad dressings made from VCO were observed in comparison with SBO. The dressings were added with TMD at various concentrations (0–3 %). Comparable colloidal stability between VCO and SBO dressings was observed. Nonetheless, higher oxidative stability of VCO samples than did SBO counterparts was markedly evident. The higher content of phenolic compounds was found for the VCO dressing. Improvement on dispersibility of the dressings could be accomplished by incorporating TMD, especially at increased concentration. TMD could also maintain pH and color changes of the dressings during a storage of 8 weeks. Comparable sensorial acceptability between the VCO and SBO dressings was observed in a presence of 3 % TMD. The present study suggested that VCO could be employed to prepare salad dressing with desirable physicochemical stability and enriched phenolic compounds.

## CHAPTER 6

## SUMMARY AND FUTURE WORKS

### 6.1 Summary

Extraction method influenced oil recovery efficiency and physicochemical characteristics of the derived virgin coconut oil (VCO). Protease aided extraction provided the oil with highest recovery yield compared to the conventional methods of fermentation and thermal cycling techniques. The greatest content of polyunsaturated fatty acids (PUFAs) was found for the VCO produced through protease aided extraction, whereas the highest phenolic compounds and tocopherol contents were present in the oils recovered through fermentation mean. Coconut fruit maturity also affected to the VCO characteristics. The coconut at intermediate- (12 months old) and old (13 months old) age ranges contained higher oil content than did the young coconuts (11 months old). However, the highest contents of phenolic compounds and tocopherol were found for the young coconuts.

When VCO was employed as a dispersed phase to prepare a model oil-in-water (O/W) emulsion, it was found that VCO could provide the emulsions with greater colloidal and oxidative stabilities compared to the soybean oil (SBO) emulsions, especially at the increased oil volume fraction. The present results suggested that VCO could be promisingly used to prepare concentrated emulsions with appreciable physicochemical stability.

To utilize VCO in emulsified food product, salad dressing was selected as a food model. The dressings were prepared using VCO and tapioca maltodextrins (TMD) at different concentrations (0–3%, w/w) as a stabilizer. Properties of the VCO dressings were monitored in parallel with the SBO dressings. Improvement on colloidal stability of the dressings could be achieved by incorporating TMD, especially at the increased concentrations, suggested by lowered emulsion size increasing and creaming rate of the TMD added samples than the control (dressings without TMD) along 8 weeks. TMD, moreover, could retard pH change and retain color of the dressing during storage. By using VCO, the dressings with better oxidative stability compared to the SBO

counterparts could be received. Higher total phenolic content was observed for the VCO dressings than those found for SBO dressings, suggesting to a better stability of the former sample. Difference on colloidal stability and sensory acceptance between VCO and SBO dressings was not noticeable. The present work indicated that VCO could be potently employed to prepare salad dressing with desirable characteristics, especially in a presence of TMD.

## 6.2 Future works

Study on utilization of VCO in other food products (*e.g*., ice cream) should be implemented. Considering on higher polarity and better oxidative stability than other seed oils, VCO may be a suitable carrier for some bioactive compounds to be introduce in functional food formulation. With capacity to enhance stability of emulsified model, moreover, TMD may be a useful stabilizer for other emulsified foods.

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**List of Publication**

1. Prapun, R., Cheetangdee, N. and Udomrati, S. 2016. Characterization of virgin coconut oil (VCO) properties recovered by different techniques and fruit maturities. Int. Food Res. J. *In Pressed.*

**List of Proceeding**

1. Prapun, P. and Cheetangdee, N. 2015. Physicochemical stability of oil-in-water emulsions consisting of different oil types. The 41$^{st}$ congress on science and technology of Thailand (STT 41). Conference. Nakhon Ratchasima, Thailand. 6-8 November, 2015. Poster presentation.