FORMULATION DEVELOPMENT AND SHELF LIFE STUDY OF MACADAMIA OIL BLENDED WITH PERILLA OIL AND SACHA INCHI OIL FOR BALANCED OMEGA 6:3 FOOD SUPPLEMENTS

JIRATCHAYA JUDPHOL

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science in Food Science and Technology - (Type A 2)
2021

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By JIRATCHAYA JUDPHOL

has been approved by the Graduate School as partial fulfillment of the requirements for the Master of Science in Food Science and Technology - (Type A 2) of Naresuan University

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Title	FORMULATION DEVELOPMENT AND SHELF LIFE STUDY OF MACADAMIA OIL BLENDED WITH PERILLA OIL AND SACHA INCHI OIL FOR BALANCED OMEGA 6:3 FOOD SUPPLEMENTS
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ABSTRACT

From literature review macadamia oil (MO) had higher omega 6:3 ratio (38.5:1) than the recommendation (4-10:1) by World Health Organization (WHO) while perilla oil (PRO) and sacha inchi oil (SIO) had much lower omega 6:3 ratio (0.2:1 and 0.6:1, respectively). The objective of this study was to investigate the suitable blending ratio of MO with PRO and SIO to have the ratio of omega 6:3 close to the recommended level by WHO. In this study, each parental oil was analyzed for their properties including physical, chemical, and nutritional properties. MO had higher L* and viscosity but lower a*, b*, acid value, antioxidant activity and nutritional properties than that of PRO and SIO. Parental MO exhibited omega 6:3 ratio of 11.57, while PRO and SIO were 0.31 and 1.08, respectively. Fatty acid composition, particularly the ratio of omega 6:3 of each parental oil was analyzed and used to calculate for a blending ratio, aimed to get a balance omega 6:3 ratio, closest to 10. MO was blended with 6 different ratios of each PRO and SIO. Blending at different ratios of MO:PRO and MO:SIO showed significantly different ($p<0.05$) in physical, chemical, and nutritional properties. The suitable blending ratio of MO with PRO and SIO were 190:1 and 100:1 with omega 6:3 ratio of 9.98 and 9.56, respectively. These ratio were chosen for developing a food supplement in a form of oil capsule and studied for the changes during storage at room temperature for 120

days in three different types of packages including white plastic, amber plastic, and amber glass bottle. Different types of packages and storage time significantly affected the physical, chemical and nutritional properties of both MO:PRO and MO:SIO capsules ($p<0.05$). After storage for 120 days, acid value and antioxidant activity of both MO:PRO and MO:SIO capsules were decreased while peroxide value and thiobabituric acid value were increased. However, the values were conformed to the standard of CODEX. The production cost of MO:PRO capsules at ratio of 190:1 was 106 Baht/bottle, whereas MO:SIO at ratio of 100:1 was 109 Baht/bottle (60 capsules packed in amber glass bottle). Price differences were mainly contributed by differences in the price of raw materials used. Data from this study indicated that macadamia industry could use either PRO or SIO to blend with MO in order to improve its omega 6:3 ratio.

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CHAPTER I

INTRODUCTION

Background

Omega fatty acids are unsaturated fatty acids as they contain at least one double bond in their structures. Omega fatty acids can be either monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) depending on the number of double bond in their structures. Oleic acid (omega 9) is one of omega fatty acids that classified as MUFA while linoleic acid (omega 6) and linolenic acid (omega 3) are omega fatty acids that classified as PUFA.

Linoleic acid (omega 6) and linolenic acid (omega 3) are essential fatty acids that cannot be synthesized by human, only obtain from human diets. They are commonly found in fatty fish like salmon or mackerel, soybean, nut and seeds. Both omega 6 and omega 3 are considered as a healthy fat due to their healthy benefits. When they are consumed by human, they are changed to eicosapentaenoic (EPA) (Simopoulos, 2016) which is helped in improving the human growth, brain functions, and digestion system. However, the World Health Organization (WHO) recommends the proper ratio of omega 6:3 fatty acids of 4-10:1. This proper ratio will enhance thermogenesis, building muscle and hormones such as eicosanoids, and improving brain functions (Mostafa et al., 2013). However, intake of higher ratio of omega 6:3 fatty acid increases the prevalence of overweight or obesity (Simopoulos, 2016), and promotes the pathogenesis of cardiovascular disease, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2008; DiNicolantonio, & O'Keefe, 2018).

Macadamia (*Macadamia integrifolia*) is indigenous crop of Australia that was found since 1843. Macadamia nut fruit is composed of husk, shell and kernel. Macadamia kernel is an edible part that mostly consumed as roasted or fried, and also used as the ingredient in many confectionary products. Nowadays, macadamia becomes the important commercial crops. The demand of macadamia in world market has been increasing continuously and the biggest producer of macadamia comes from Australia with approximately 14,100 metrics tons (Navarro, & Rodrigues, 2016).

Due to an increasing of demand, macadamia has been growing more in many countries including Thailand.

Macadamia becomes more popular due to its taste and health benefits. Macadamia contains fat approximately 70% (Laohasongkram et al., 2011), 75% (Birch et al., 2010), or 78% (Navarro, & Rodrigues, 2016). Most of the fat in macadamia is unsaturated fatty acid that is considered as a healthy fat. Unsaturated fatty acid is possible to lower blood cholesterol, and reduce the risk of heart disease (Maguire et al., 2004; Jitngarmkusol et al., 2008; Laohasongkram et al., 2011; Rudzińska et al., 2016; Hashempour-Baltork et al., 2018). Even though macadamia kernel contains high amount of healthy fatty acids, the ratio of omega 6:3 fatty acids in macadamia kernel is up to 38.5:1 (Aquino-Bolaños et al., 2017) or 24.75:1 (Kaijser et al., 2000). This indicates much higher amount of omega 6 fatty acid than omega 3 fatty acid. In order to reduce the ratio of omega 6:3 fatty acids in macadamia, the rich omega 3 fatty acid oil is needed for blending with macadamia oil.

Many plant oils that are rich in omega 3 fatty acid such as linseed oil 57.21% (Hashempour-Baltork et al., 2018), chia oil 64.75% (Ayerza, & Coates, 2011), perilla oil 58% (Asif, 2011) and sacha inchi oil 47% (Maurer et al., 2012). This research focused on using perilla oil and sacha inchi oil for blending with macadamia oil due mainly to their availability in Thailand.

Therefore, this research was aimed to formulate the macadamia oil blended with perilla oil and sacha inchi oil to have more proper ratio of omega 6:3 fatty acids as close to the recommended ratio by WHO as possible. To be more convenient to consume in an adequate amount and easy to carry along, the blended oils were packed in capsules and considered as food supplement. In addition, the blended oils properties' data were beneficial for further utilization by the macadamia industry.

Research objectives

1. To extract the oil from macadamia, perilla and sacha inchi and study its properties and fatty acid composition

2. To formulate and analyze the quality of macadamia oil blended with perilla oil and sacha inchi oil with a balancing ratio of omega 6:3

3. To produce and investigate the shelf life and suitable package of the blended oil capsules

4. To calculate the production cost of blended oil in the form of capsules packing in a suitable packaging

CHAPTER II

LITERATURE REVIEW

Omega fatty acids

Omega fatty acids are type of fatty acid that classified as unsaturated fatty acids. They are fatty acids containing one or more double bonds. Double bond in structure of unsaturated fatty acid can be occurred in *-cis* or *–trans* configuration. The amount of double bond leads to categorization of omega fatty acids into MUFA and PUFA.

MUFAs are fatty acids contain double bond only one position. The position of double bond in their structures makes the difference among them. Important omega fatty acids that classified as MUFA are omega 9 (oleic acid) and omega 7 (palmitoleic acid, vaccenic acid, and paulinic acid).

PUFAs contain two or more double bond in their structures. The different position of double bond makes them different from each other as well. Omega 6 (linoleic acid, arachidonic acid, adrenic acid, etc.) and omega 3 (linolenic acid, stearidonic acid, eicosatetraenoic acid, etc.) are the most important PUFAs as omega fatty acids. However, among the omega 6 and omega 3 fatty acids, linoleic acid (omega 6) and linolenic acid (omega 3) are the most abundant found in macadamia (Kaijser et al., 2000; Maguire et al., 2004) which involve with this research.

Linoleic acid (Omega 6)

Linoleic acid (18:2, ω 6; cis, cis-9, 12-octadecadienoic acid) or omega 6 is a polyunsaturated omega 6 fatty acid. It is consisted of 18 carbon atoms with two double bonds (Figure 1). It can be used as a source of energy. Linoleic acid functions as a structural component to maintain a certain level of membrane fluidity of the transdermal water barrier of the epidermis (Whelan, & Fritsche, 2013). Linoleic acid can be found in various oil such as sesame oil, soybean oil, corn oil, sunflower oil, safflower oil, almond oil and fish oil. The consumption of seed oils high in the omega 6 is possible to contribute a low-grade inflammation, oxidative stress, endothelial dysfunction and atherosclerosis (DiNicolantonio, & O'Keefe, 2018).

Linolenic acid (Omega 3)

Linolenic acid (18:3, ω 3, a*ll-cis*-9, 12, 15-octadecatrienoic acid) or omega 3 is a polyunsaturated omega 3 fatty acid. It is consisted of 18 carbon atom with three double bonds (Figure 1). It is mostly found in vegetable oil such as soybean oil, canola oil and flaxseed oil.

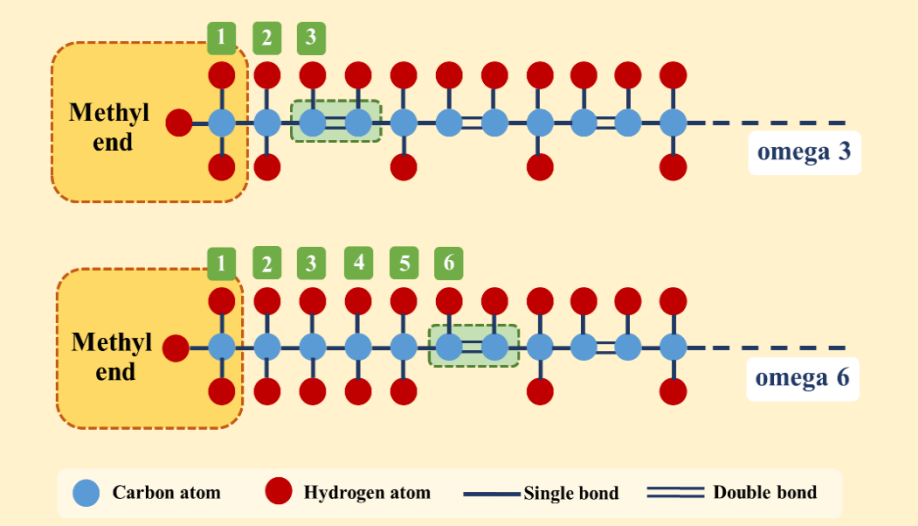

Figure 1 Structure of linolenic (omega 3) and linoleic (omega 6) acid

Ratio of omega 6 and omega 3 fatty acids

The WHO recommends the proper ratio of omega 6:3 ranged between 4-10:1 (Mostafa et al., 2013). Most of the human diets has omega 6:3 high at 15-16.7:1, especially Western diets (Simopoulos, 2002).

The diets with imbalance of omega 6 and omega 3 ratio leads to health effects in various ways. There is a report that too high ratio of omega 6:3 could promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases. High omega 6 intake, increases the risk factor for those diseases and can be decreased by intake of omega 3 (Simopoulos, 2002).

Moreover, increasing the ratio of omega 6:3 shows an increase in the risk of obesity. Since high omega 6 and high omega 6:3 intake are associated with weight

gaining while high omega 3 intake are able to reduce the risk of weight gaining (Simopoulos, 2016). When omega 6 is intaken, it will be metabolized to arachidonic acid (C20:4, ω-6). This arachidonic acid plays important role for pre-adipocyte becoming mature adipocyte and resulting in weight gain that will be inhibited by omega 3. Arachidonic acid also has ability to reduce fat deposition in adipose tissue (Ukropec et al., 2003).

On the other hand, consuming the diets with proper ratio of omega 6:3 fatty acid especially the oil sources diet will help in enhancing thermogenesis, building muscles, and hormones like eicosanoids, reducing risk factors of cardiovascular disease, high levels of blood pressure, triglycerides, lipoprotein–a, fibrinogen, clot formation and inflammation, muscle breakdown, improving brain functions, mood, intelligence, behavior and vision (Mostafa et al., 2013). Moreover, there is a report that proper ratio of omega 6:3 be able to prevent cardiovascular disease, reduce rectal cell proliferation, reduce the risk of breast cancer in women, as well as reduce the risk of many of the chronic diseases (Simopoulos, 2002). It can be concluded that omega 6:3 of dietary should be kept at low.

To keep omega 6:3 of dietary to be low, consumers can change the dietary source from high in omega 6 to high in omega 3 or MUFA instead. Such as change from consuming soy bean oil that contains high omega 6 to perilla oil or olive oil that contain high omega 3 and MUFA, respectively. Moreover, mixing or blending of rich in omega 3 to omega 6 dietary to balance omega 6:3 of final dietary is one of alternative ways to prevent negative health effect of high omega 6:3.

Macadamia

Macadamia refers to the four types of plant species that are native to the New South Wales and Queensland states of Australia, including *Macadamia integrifolia, Macadamia jansenii, Macadamia ternifolia,* and *Macadamia tetraphylla*. Two species, *M. integrifolia*, & *M. tetraphylla*, are of commercial importance for their nuts while the other two, *M. jansenii* and *M. ternifolia* are toxic due to high content of cyanogenic glycosides (Hu et al., 2019). Macadamia nut fruit is composed of husk, shell and kernel. The kernel of macadamia is only one part that is edible. There is a report that the flavor of macadamia kernel is related to the oil content, volatile

constituents and possibly to carbohydrates, especially sugars. Moreover, macadamia will be greater flavor rating when the oil content is greater. The macadamia kernel quality is primarily indicated by appearance including size, shape, color, texture, freedom from defects and shelf life or storability (Buthelezi et al., 2019).

After the macadamia nuts are harvested from the tree, removing of the fibrous pericarp or husk is the first postharvest operation. After this step, macadamia nuts, nut-in-shell (NIS), are dried at farm to reduce the moisture content to around 10%. Then they are finally dried to approximately 3% moisture content. Finally, they are cracked for the kernels for further process of different products (Walton, & Wallace, 2011).

Macadamia kernels utilization

Macadamia is used in various purposes and industries. Mostly macadamia is purchased in the form of roasted or fried as snack and chocolate-covered candy. Roasting macadamia improves flavor, aroma, color, texture and appearance. Moreover, roasting process is able to preserve quality and improve storability of macadamia as well (Buthelezi et al., 2019). Macadamia is also used as ingredient in some food manufactures especially ice-cream and baking industry. Macadamia kernel is able to extracted oil due to high content of oil. Besides using as a cooking oil, macadamia oil can also be used as ingredients for cosmetic industry.

Macadamia kernel composition

Macadamia is considered as a healthy nut due to its nutrition. It is one rich source of energy due to high content of oil. Macadamia from different sources contains different compositions depending on the species and its growing area. However, the main composition of macadamia is always fat (lipid or oil) even from any source. The composition of macadamia is shown in Table 1.

Table 1 Composition of macadamia kernel

Composition	Content (%)
Moisture	3-11
Lipids	33-65
Protein	8-20
Crude fiber	6-30
Ash	1-2

Source: Navarro, & Rodrigues, 2016

As macadamia contain high content of fat that consists various types of fatty acid. Fatty acid composition of macadamia kernel is different depending on the species and growing area. The main fatty acid that could be found in macadamia is oleic acid (omega 9), as shown in Table 2. Macadamia kernel contains linoleic and linolenic acids as well. All of these fatty acids are considered as healthy fatty acids due to their healthy benefits to human health as previously described. However, the ratio of omega 6:3 in macadamia kernel is too high, up to 24.75:1 or 38.5:1 that is much higher than the recommended ratio from WHO (4-10:1) (Mostafa et al., 2013).

Table 2 Some fatty acid composition of macadamia kernel

Type of fatty acid	Content (g/100 g of fat)		
	Maguire et al. (2004)	Kaijser et al. (2000)	Hu et al. (2019)
Palmitic acid, C16:0	8.37	8.50	6.0-9.5
Palmitoleic acid, C16:1 (ω-7)	17.28	21.50	15.0-22.0
Stearic acid, C18:0	3.17	1.49	2.0-5.5
Oleic acid, C18:1 (ω-9)	65.15	57.17	55.0-67.0
Linoleic acid, C18:2 (ω-6)	2.31	3.96	3.0
Linolenic acid, C18:3 (ω-3)	0.06	0.16	1.0-5.5
Arachidoic acid, C20:0	2.28	1.55	0.1-3.0
Ratio of linoleic acid and linolenic acid (ω -6: ω -3)	38.5	24.75	1.5-3.5

Macadamia oil extraction

The most critical step that affects the quality and quantity of extracted oil from kernels or seeds is the extraction step. The macadamia oil extraction process can be carried out in different ways as described below.

1. Solvent extraction

Solvent extraction is a process that transports mass from one phase to another. The liquid solvent comes into contact with the lipid matter in macadamia kernels, interacting with it and subsequently dragging the lipids out of the solid matrix. Solvent, such as hexane, toluene, and ethyl acetate, has a potential for extraction. Organic solvents in the food industry have some disadvantages, such as health and safety problems due to their properties. It is flammable and toxic. Therefore, it is able to causes adverse environmental effects (Cheng et al., 2011). There are several more ecology and environmentally friendly extraction techniques available over the solvent extraction such as supercritical fluid extraction and hydraulic and screw presses.

2. Supercritical fluid extraction

Supercritical fluid extraction is the process of separating one component from another by using supercritical fluids as extracting solvent. The super critical fluids are the gas beyond its critical temperature and pressure. Therefore, it has both the properties of gas and liquid, as it has density like liquid and viscosity like gas that enhances the diffusivity of the fluid in the product-containing matrix and increases the rate of extraction of the targeted compound. Although supercritical fluid extraction is a green technology and is capable of extracting higher quality oil, it is still very expensive to use on a large scale. Thus, it is not the best option to extract good quality oils at an affordable price (Ribeiro et al., 2020).

3. Cold pressing technique

Traditionally, macadamia oil extraction is carried out by cold pressing (Navarro, & Rodrigues, 2016). Cold pressing method can be done by screw pressing or hydraulic pressing. Both are considered adequate methods of mechanical extraction for small and medium scale production levels since it is environmentally friendly. It is operated by applying pressure to the oleaginous material and keeping the temperature less than 30°C (Kamal-Eldin, & Moreau, 2009).

The disadvantage of mechanical extraction methods is that they provide a lower yield when compared to solvent methods of oil extraction (Santoso, & Inggrid, 2014). However, cold pressing technique is safe and more suitable to extract functional oils for the dietary supplement as it able to maintain important compounds than other extraction technique and no use of any chemical. Therefore, it was used in this study.

Macadamia oil utilization

Macadamia oil is widely used in many purposes. It is possible to use as cooking oil due to its unique physical and biochemical properties such as having a high smoke and flash point (200°C and 300°C, respectively). Moreover, macadamia oil has important role in term of cosmetic applications as it contains phytosterols and linoleic acid, that aids skin recovery, retains moisture, and prevents inflammation (Navarro, & Rodrigues, 2016; Hu et al., 2019).

Perilla seed

Perilla frutescens L. belongs to the family *Lamiaceae* P. Frutescens, commonly known as perilla. It is an annual herb cultivated for its edible seeds that found in China, Korea, Japan and the Himalayan region of India and Nepal (Dhyani et al., 2019). Perilla is becoming popular due to a growing economic importance. It is also being cultivated by many western countries like U.S.A, Russia, Europe, as well as Thailand.

Perilla utilization

The plant of perilla both leaf and seed is very useful and nutritious as it contains fat, protein, vitamins, minerals and phytochemicals. Perilla leaves are used as vegetable and spices as well to impart color and flavor in many dishes while the seeds are mostly used as ingredient for various dishes. In Korea and Japan, both leaves and seeds are used for adding more flavor. Moreover, the seeds is extracted oil and used as condiment oil in form of powder or oil (Yu et al., 2017; Dhyani et al., 2019).

Perilla seed composition

Proximate content of perilla seed is summarized in Table 3 that showed the main component of perilla seeds is lipid. Therefore, perilla is one alternative plant

that has a potential to be extracted oil. Perilla seeds also have been found to contain some essential minerals (calcium, iron, zinc, magnesium and phosphorous).

Table 3 Composition of perilla seeds kernel

Composition	Content (%)
Moisture	6.02
Lipids	42.27
Crude protein	25.38
Carbohydrate	23.00
Ash	3.33

Source: Dhyani et al., 2019

The major fatty acids in perilla seed is linolenic acid (omega 3) as shown in Table 4. The ratio of omega 6:3 in perilla seeds is lower than in macadamia kernels. The ratio is around 0.2-0.3:1 (Siriamornpun et al., 2006; Ding et al., 2012). However, it is lower than the recommended ratio of 4-10:1 from WHO (Mostafa et al., 2013).

Table 4 Some fatty acid composition of perilla seeds

Type of fatty acid	**Content** (g/100 g of fat)			
	Ding et al. (2012)		Siriamornpun et al. (2006)	
Palmitic acid, C16:0	7.23	6.86	7.23	6.86
Palmitoleic acid, C16:1 (ω-7)	-	0.11	-	0.11
Stearic acid, C18:0	2.89	3.16	2.89	3.16
Oleic acid, C18:1 (ω-9)	20.77	11.55	20.77	11.55
Linoleic acid, C18:2 (ω-6)	10.54	18.45	10.54	18.45
Linolenic acid, C18:3 (ω-3)	52.58	59.84	52.58	59.84
Arachidonic acid, C20:0	-	-	-	-
Ratio of linoleic acid and linolenic acid(ω-6: ω-3)	0.2	0.3	0.2	0.3

Note: (-) means data not available

Sacha inchi

Sacha inchi (*Plukenetia volubilis* L.), also named "Inca peanut", "wild peanut", "Inca inchi" or "mountain peanut", is a plant of the Euphorbiaceae family. It is native to Peru. However, it is now widely cultivated in the south of Colombia (Fanali et al., 2011). The sacha inchi plant produces star-shaped green fruits that yield edible dark brown seeds, slightly enlarged in the center and squashed toward the edges.

Sacha inchi utilization

Sacha inchi, mostly kernel is extracted oil and used as edible oil same as other vegetable oil like olive, avocado, wheat germ, and rice bran, and argan oils, and is valued for its useful physicochemical properties and good sensory attributes in terms of taste and flavor. Moreover, sacha inchi oil is also used as ingredient for cosmetic product, pharmaceutic product, and biodiesel (Fanali et al., 2011; Maurer et al., 2012; Wang et al., 2018).

Sacha inchi kernel composition

The amount of each chemical composition of the sacha inchi kernel is shown in Table 5. As the sacha inchi kernel composition is mainly fat, even lower than macadamia kernel and perilla seeds, it is still has the potential to be extracted oil.

Table 5 Composition of sacha inchi kernels

Composition	Content (%)
Moisture	3.3-8.32
Lipids	33.4-54.3
Protein	24.2-27.0
Carbohydrate	13.4-30.9
Ash	2.7-6.46

Source: Wang et al., 2018

Fatty acid composition in sacha inchi kernel is mainly linolenic acid like perilla seeds. As shown in Table 6, linolenic acid (omega 3) is higher than linoleic acid unlike macadamia kernel, the ratio of omega 6:3 in sacha inchi kernel is low, 0.6-0.7 that is lower than the recommended from WHO like perilla seeds.

Table 6 Some fatty acid composition of sacha inchi kernels

Type of fatty acid	**Content (**g/100 g of fat**)**	
	Gutiérrez et al. (2011)	**Fanali et al. (2011)**
Palmitic acid, C16:0	4.4	4
Palmitoleic acid, C16:1 (ω-7)	-	-
Stearic acid, C18:0	2.4	3
Oleic acid, C18:1 (ω-9)	9.1	9
Linoleic acid, C18:2 (ω-6)	33.4	36
Linolenic acid, C18:3 (ω-3)	50.8	47
Arachidonic acid, C20:0	-	-
Ratio of linoleic acid and linolenic acid (ω-6:ω-3)	0.6	0.7

Note: (-) means data not available

Blended oil

Nowadays, there are many available edible pure oils in the market such as soybean oil, palm oil, olive oil, sunflower oil, coconut oil, etc. However, these available single oils are not fully healthy in the consumer opinion. They start looking for edible oil that healthier than pure oil. Therefore, various blended oils were studied due to different purposes as summarized in Table 7. However, most of the studies were aimed to increase the stability of origin oil and improve the concentration of some important fatty acids.

Table 7 Previous studies on of blended oil

Type of oil	Purpose of blending	Result	Reference
Camelina oil and fish oil	Improve stability of fish oil	- Oxidative stabilities of tuna oil and salmon oil were not greatly affected by blending with camelina oil - Odor scores were significantly improved	Eidhin and O'Beirne (2010)
Flaxseed oil, olive oil and canola oil	Improve fatty acid composition	- All formula contained fatty acid composition and ratio of omega 6:3 conform to the recommended value by WHO	Mostafa et al. (2013)
Rapeseed oil with black cumin and rice bran oil	Improve nutritional quality and stability of rapeseed oil	- Rapeseed oil reached the target in raising and improving the nutritional and stability faster than the original one at different concentrations of black cumin oil and rice bran oil	Rudzińska et al. (2016)
Sesame oil and olive oil with linseed oil	Improve nutritional quality and oxidative stability of edible oil	- Blending linseed oil with sesame and olive oils created a positive nutritional effect with improved stability in formulated oils - Blended oil provided a functional oil with a balanced omega 6:3 ratio, positive levels of bioactive compounds and suitable stability	Hashempour-Baltork et al. (2018)

Some of the blended oil products, especially cooking oil, has been already launched in the market. Table 8 summarizes the information of blended oil products including the type of oil used for blending, selling point, brand and producer. This information was gathered by a market survey performed by the researcher.

Table 8 The commercial blended oil available in market

Type of oil for blending	Selling point	Brand	Producer
Palm oil and canola oil	Palm oil with increasing of omega 3 and 6 content	- Emerald	Sime Darby Oils Morakot Public Co., LTD., Thailand
Palm oil and soy bean oil	Palm oil with reducing saturated fatty acid	- Emerald and Morkot lite	Sime Darby Oils Morakot Public Co., LTD., Thailand
Canola oil and sunflower oil	Cooking oil with high content of omega 3 and 6	- Emerald	Sime Darby Oils Morakot Public Co., LTD., Thailand
		- Naturel	Lam Soon (Thailand) Public Co., LTD.
Canola oil and soy bean oil	Cooking oil with high content of omega 9	- Emerald	Sime Darby Oils Morakot Public Co., LTD., Thailand

Beside the blended oil for cooking, there are also blended oil capsules available in the market. The blended oil capsules are usually contained more than two types of oil and have different selling points. Table 9 shows the surveyed information of blended oil capsules including the type of oil used for blending, selling point, brand and producer performed by the researcher.

Table 9 The commercial blended oil capsules available in the market

Type of oil for blending	Selling point	Brand	Producer
Coconut oil and garlic oil	Improve immune system, reduce cholesterol and blood pressure	IMMOR	Immortal Corperation Co., LTD.

Type of oil for blending	Selling point	Brand	Producer
Rice bran oil and rice germ oil	High antioxidant, reduce cholesterol, and control blood sugar level	VISTRA	NDB Healthcare Co., LTD.
Black sesame oil and rice bran oil	Improve human bone, heart and brain	Suparposoth	JSP Pharmaceutical Manufactory (Thailand) Co., LTD.
Rice bran oil, sesame oil, coconut oil, olive oil, fish oil, perilla oil, garlic oil, and sacha inchi oil	High antioxidant		
Coconut oil, perilla oil, rice bran oil, and garlic oil	Improve blood circular system		
	Reduce risk of heart disease, high blood pressure, and diabete	Multi 4 oil	Nutrition Profess Co., LTD.
Coconut oil, black sesame oil, perilla oil, garlic oil, and avocado oil	Improve and balance burning system of human	Rai-Tai	G-Gen Group Co., LTD.
Black sesame oil, rice bran oil, and perilla oil	Improve human bone and eye		
Coconut oil, perilla oil, garlic oil, rice germ oil, flaxseed oil, and sesame oil	Reduce cholesterol on human blood, blood pressure, and risk of Alzheimer's disease	Nuti-Master	F.C.P. Co., LTD.
Black sesame oil, perilla oil, rice bran oil, coconut oil, and avocado oil	Improve human heart and bone, and reduce cholesterol in human blood	MIX NINE	Duangdee Co., LTD.
Hemp seed oil, coconut oil, rice bran oil, safflower oil, perilla oil, sacha inchi iol, flaxseed oil, black sesame oil, garlic oil, and baby jackfruit oil	Improve brain function, human bone, and immune system	Amerprai	Amado Group Co., LTD.

Blended oil capsules available in the market are mostly blended for human health benefits and consumed as food supplement. Although there are many types of oil used for making blended oil capsules, there is no macadamia oil used for blending yet. Therefore, this research is aimed to develop macadamia oil to be food supplement capsules. However, macadamia oil is high in omega 6:3 ratio. Consequently, in this study macadamia oil was blended with perilla oil and sacha inchi oil to balance omega 6:3 of macadamia oil.

CHAPTER III

RESEARCH METHODOLOGY

Materials

Macadamia kernels harvested in August 2021, used in this study were supported by Asian Stainless Co., LTD., Thailand while perilla seeds harvested in November and sacha inchi NIS harvested in August 2021, were purchased from farmers in Thailand.

Methodology

The methodology was divided into 4 parts; 1) Oil pressing and investigating oil properties and fatty acid composition, 2) Blending oil for a balancing ratio of omega 6:3, 3) Capsule packing and shelf life study of blended oil, and 4) Production cost calculation of blended oil in capsules. The overall methodology was illustrated as shown in Figure 2.

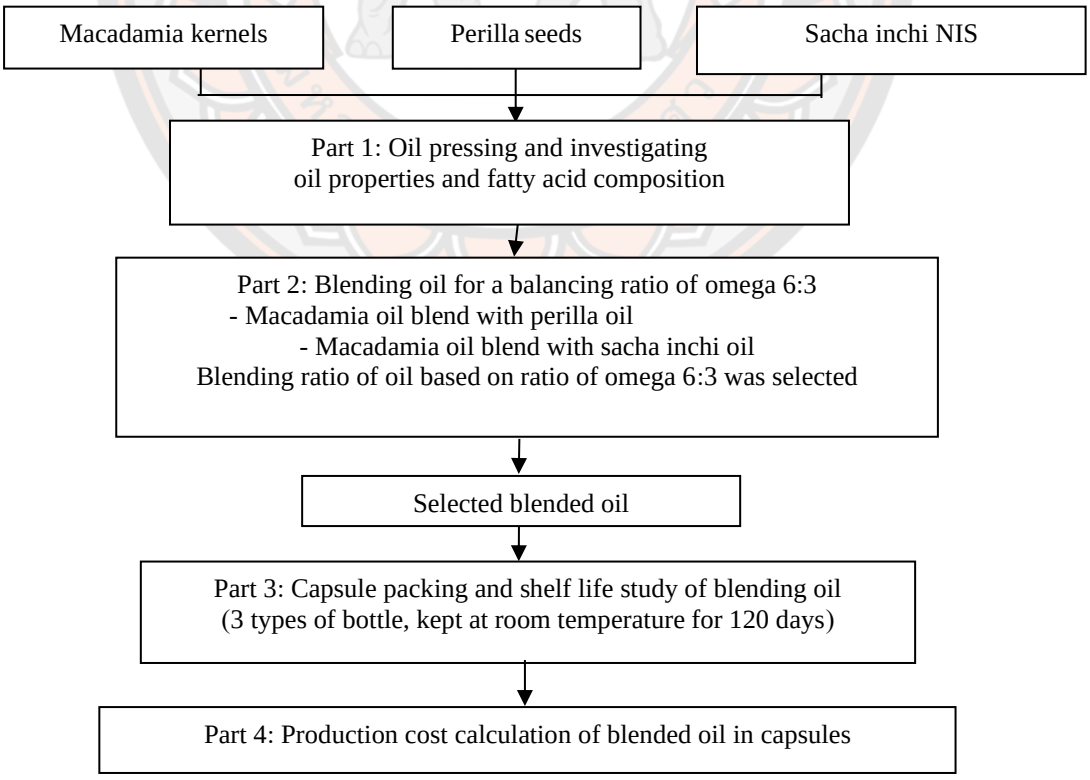

Figure 2 Flow chart of research methodology

Part 1 Oil pressing and investigating oil properties and fatty acid composition

Broken and under sized macadamia kernels, and sacha inchi nut-in-shell (NIS) were pressed for oil by using a cold-pressing technique with a 2 screw type expellers whereas perilla seed were pressed using a desktop pressing machine (Figure 3). The suitable extraction conditions were adjusted for gathering a maximum oil yield for each sample. The extracted oils were analyzed for the following properties (details were described in appendix I):

1. **Physical properties**

1.1 Color measurement by colorimeter Minolta model CR-100, USA

1.2 Viscosity measurement by Brook-field Viscometer (RVDV ll, Model 845X, USA)

2. **Chemical properties**

2.1 Fatty acid composition (In house method TE-CH-208 based on AOAC, 2012)

2.2 Acid value (AV) (AOCS, 2004)

2.3 Peroxide value (PV) (AOCS, 2004)

2.4 Antioxidant activity (DPPH and ABTS assays) (Anagnostopoulou et al., 2006)

2.5 Thiobarbituric acid value (TBA value) (Vasile et al., 2019)

3. **Nutrition properties** (Hashempour-Baltork et al., 2016)

3.1 Atherogenic index (AI)

3.2 Thrombogenic index (TI)

3.3 Hypocholesterolemic: hypercholesterolemic (HH) ratio

3.4 Polyunsaturated fatty acid: saturated fatty acid (PUFA-SFA) ratio

3.5 Linoleic-Linolenic (omega 6:3) ratio

All the nutritional properties were calculated from the fatty acid composition data (as shown in appendix I).

(A)

(B)

Figure 3 Cold-pressing machine for macadamia kernels, sacha inchi NIS (A), and perilla seeds (B)

Part 2 Blending oil for a balancing ratio of omega 6:3

The extracted macadamia oil from Part 1 was blended with extracted perilla oil and sacha inchi oil from Part 1 at different ratios. Macadamia oil was blended with perilla oil for 6 ratios and with sacha inchi oil for 6 ratios. Blending method was done as described by Abdel-Razek et al. (2011) with some adjustments. Blended oil 200 g was placed in a 600 ml beaker before blending by using 8 cm magnetic bar and stirred at 640 rpm for 5 min. The blended oil was analyzed for the following properties (details were described in appendix I):

1. **Physical properties**

1.1 Color measurement by colorimeter Minolta model CR-100, USA

1.2 Viscosity measurement by Brook-field Viscometer (RVDV ll, Model 845X, USA)

2. **Chemical properties**

2.1 Fatty acid composition (In house method TE-CH-208 based on AOAC, 2012)

2.2 Acid value (AV) (AOCS, 2004)

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3. Nutrition properties (Hashempour-Baltork et al., 2016)

3.1 Atherogenic index (AI)

3.2 Thrombogenic index (TI)

3.3 Hypocholesterolemic: hypercholesterolemic (HH) ratio

3.4 Polyunsaturated fatty acid: saturated fatty acid (PUFA-SFA) ratio

3.5 Linoleic-Linolenic (omega 6:3) ratio

All the nutrition properties were calculated from the fatty acid composition data.

After the physical and chemical analyses, the blended oils were selected based on the ratio of omega 6:3 closest to 10:1. One formula was selected from each blending between macadamia and perilla oil, and macadamia and sacha inchi oil.

Moreover, to determine the correlation of each chemical and physical properties among the samples, the exploratory principal component analysis (PCA) was employed at a confidence level of 95%.

Part 3 Capsule packing and shelf life study of blended oil

1. Capsule packing of blended oil

The 2 selected blended oils from Part 2 were filled in hard capsules and sealed. Blended oil (500 g) was filled in each hard capsule as shown in Figure 4. These capsules were further packed in 3 different types of bottle and used for the shelf life study.Three different types of bottle were white plastic (PE), amber plastic (PET), and amber glass bottle. The capsule filling machine was shown in Figure 5 and the different types of bottle were illustrated in Figure 6.

Figure 4 Blended oil in capsules

Figure 5 Capsule filling machine

(A) (B) (C)

Figure 6 Three different types of bottle (white plastic bottle (A), amber plastic bottle (B), and amber glass bottle (C))

2. Shelf life study of blended oil in capsules

For shelf life study, the 60 blended oil in capsules were packed in three different packages including white plastic bottle, amber plastic bottle, and amber glass bottle and kept in dark place at room temperature for 120 days. The samples were randomly selected for properties analysis at day 0, 30, 60, 90 and 120 to regulate the changes in their properties. The analysis included (details were described in Appendix I):

2.1 Color measurement by colorimeter Minolta model CR-100, USA

2.2 Acid value (AV) (AOCS, 2004)

2.3 Peroxide value (PV) (AOCS, 2004)

2.4 Antioxidant activity (DPPH and ABTS assays) (Anagnostopoulou et al., 2006)

2.5 Thiobarbituric acid value (TBA value) (Vasile et al., 2019)

Part 4 Production cost calculation of blended oil in capsules

The production cost of blended oil in capsules per bottle (60 capsules) at a laboratory scale was calculated as a guiding for further production in a pilot scale or commercial scale by using the below formula:

Total cost = (Raw materials cost + Labor cost + Production cost) / Amount of product

Statistical analysis

All experiments were conducted in triplicates and the results were expressed as mean ± SD. The statistical examination of the data was performed, using the SPSS program. A difference of mean values was analyzed, using an analysis of the variance (ANOVA) test. These means were compared, using the Duncan's New Multiple Range Test (DMRT) and $p<0.05$ was applied, in order to establish significant differences.

The PCA was employed in order to visualize correlation of each chemical and physical properties among the samples. The PCA model was performed at a confidence level of 95%.

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CHAPTER IV

RESULTS AND DISCUSSION

This chapter reported the results of experiment and discussed the results that were divided into four parts as followed.

Part 1 Oil pressing and investigating oil properties and fatty acid composition

For part 1, macadamia oil (MO), perilla oil (PRO) and sacha inchi oil (SIO) were studied for their physical, chemical and nutritional properties. The fatty acid composition was analyzed and used for calculating the blended ratio for MO:PRO and MO:SIO, aiming to get omega 6:3 ratio of the blended oil closest to 10:1 as recommended by WHO. Macadamia kernels, perilla seeds and sacha inchi NIS were extracted for the oil by cold pressing process. The suitable condition was adjusted for each raw material. The results of three parental oil properties were shown in Figures 7-8 and Tables 10-12.

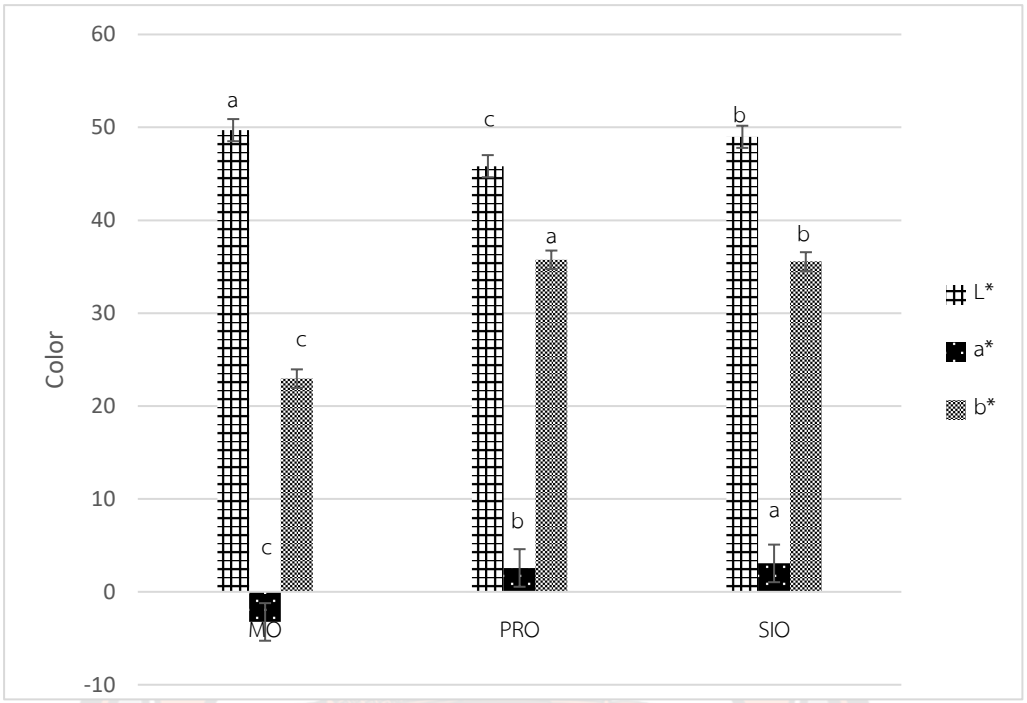


Figure 7 L*, a* and b* of MO, PRO and SIO

Note: Different letters (a-c) within a same pattern bar showed significantly different ($p<0.05$)

Table 10 Viscosity of MO, PRO and SIO at 20 rpm, room temperature

Sample	Viscosity (cP)
MO	49.62 ± 0.34^{a}
PRO	33.42 ± 0.17^{b}
SIO	32.52 ± 0.43^{c}

Note: Means with different letters (a-c) within the same column showed significantly different ($p<0.05$)

L* indicated brightness of samples, a* indicated redness (+) and greenness (-), and b* indicated yellowness (+) and blueness (-). From Figure 7, the results showed that MO had a light yellow with slightly green, while PRO and SIO showed

darker yellow with red color. This was due to they were different in their original pigments. There was the report that perilla seed contained some anthocyanin, and apigenin and luteolin that exhibited redness and yellowness, respectively (Ishikura, 1981). While sacha inchi shell contained some carotenoid and gossypol that exhibited redness and yellowness, respectively (Ramos-Escudero et al., 2019).

MO had much higher viscosity than PRO and SIO ($p<0.05$), as showed in Table 10, due to fatty acids in MO were mainly MUFA that contained only one double bond in their structures while PRO and SIO were mainly PUFA that contained more than one double bond. These double bonds caused kink in their straight chains, when kink was occurred, fatty acid molecules were not allowed to stack together (Kim et al., 2010). Consequently MO that contained less double bond, had less kink in their structures, fatty acid molecules could stack together, causing higher viscosity.

Acid value (AV) indicated the quality of oil that degraded from hydrolysis reaction. The reaction caused triglyceride degraded to free fatty acids. AV of cold pressing process must be under 4 mg KOH/g to meet the Codex standard. From the experiment, three parental oils had AV lower than 4 mg KOH/g (Table 11), conforming to standard. However, PRO and SIO had higher AV than MO due to either containing more triglycerides or having higher degree of hydrolysis. Peroxide value (PV) and thiobarbituric acid value (TBA) indicated the degradation of oil from oxidation reaction that be able to cause rancidity of oil. MO, PRO, and SIO from cold pressing process showed no PV and TBA value, indicated that there were no oxidation occurred.

The antioxidant activity both DPPH and ABTS of PRO and SIO were higher than MO ($p<0.05$) (Table 11). This might be due to during the extraction both PRO and SIO were extracted with outer shells and those shells contained some phenolic compounds such as anthocyanin, carotenoid, tocopherol and chlorophyll (Zhou et al., 2014; Štěrbová et al., 2017) that might be extracted out with oil. Whereas MO was extracted from only the kernels without any shell. Moreover, DPPH assay showed lower antioxidant activity due to the reaction of DPPH was slower than ABTS (Martysiak-Żurowska, & Wenta, 2012). Therefore, at the same limit of reaction time in measurement, antioxidant activity of DPPH occurred less than ABTS assay.

Table 11 Acid value (AV) and antioxidant activity of MO, PRO and SIO

Sample	Acid value (mg KOH/g)	Antioxidant activity (mg Trolox/g)	
		DPPH	ABTS
MO	1.95±0.00[c]	1.95±0.06[c]	12.44±0.83[c]
PRO	2.24±0.00[a]	3.04±0.08[a]	21.30±0.67[a]
SIO	2.21±0.02[b]	2.87±0.05[b]	19.05±0.94[b]

Note: Means with different letters (a-c) in a same column showed significantly different ($p<0.05$)

Fatty acid composition of MO, PRO, and SIO were shown in Table 12. There were 22 types of fatty acids found, consisting of 9 saturated fatty acid (SFA), 5 MUFA and 8 PUFA. MO contained mainly MUFA that was oleic acid (C18:1). This result was similar to previous studies (Rengel et al., 2015; Canneddu et al., 2016; Buthelezi et al., 2019). PRO and SIO contained mainly PUFA that were linoleic acid (C18:2, omega 6) and linolenic acid (C18:3, omega 3), agreeable with previous studies (Siriamornpun et al., 2006; Fanali et al., 2011; Gutiérrez et al., 2011; Ding et al., 2012;). MO contained omega 6 much higher than omega 3, resulting in higher ratio of omega 6:3 (11.57) while PRO and SIO contained higher omega 3, contributing to lower ratio of omega 6:3 (0.31 and 1.08, respectively).

Furthermore, nutritional properties of MO, PRO, and SIO were different due to differences in their fatty acid compositions. Nutritional properties included Atherogenic index (AI), Thrombogenic index (TI), Hypocholesterolemic: Hypercholesterolemic ratio (HH ratio), PUFA:SFA, and Omega 6:3. AI, TI, and HH ratio were able to use for prediction the risk of cardiovascular disease (Hashempour-Baltork et al., 2018). The desirable nutritional aspect included lower AI and TI but higher HH ratio that would indicate lower risk of cardiovascular disease. Moreover, omega 6:3 should be low while PUFA:SFA should be high. From Figure 8, it showed that MO had worse nutritional properties than PRO and SIO as it had lower desirable values of HH and PUFA:SFA ratio and higher undesirable values of AI, TI, and omega 6:3. In other words, PRO and SIO had lower risk to cardiovascular disease than MO.

Table 12 Fatty acid composition of MO, PRO and SIO

Fatty acid	Content (g/100 g of fat)		
	MO	PRO	SIO
1. Lauric acid (C12:0)	0.06	ND	ND
2. Myristic acid (C14:0)	0.66	0.02	0.01
3. Palmitic acid (16:0)	8.1	7.48	4.85
4. Palmitoleic acid (C16:1n7)	11.11	0.22	0.05
5. Heptadecanoic acid (C17:0)	0.03	0.04	0.08
6. cis-10-Heptadecenoic acid (C17:1n10)	0.06	0.02	0.04
7. Stearic acid (C18:0)	4.35	3.02	3.46
8. trans-9-Elaidic acid (C18:1n9t)	0.04	0.03	0.03
9. cis-9-Oleic acid (C18:1n9c)	51.56	11.82	13.87
10. cis-9,12-Linoleic acid (C18:2n6)	18.27	18.06	39.87
11. gamma-Linolenic acid (C18:3n6)	ND	0.04	0.18
12. alpha-Linolenic acid (C18:3n3)	1.21	58.61	37.2
13. Arachidic acid (C20:0)	2.1	0.2	0.18
14. cis-11-Eicosenoic acid (C20:1n11)	1.41	0.24	0.03
15. cis-11,14-Eicosadienoic acid (C20:2)	0.03	0.04	ND
16. cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.02	0.03	ND
17. cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.16	0.03	0.01
18. Arachidonic acid (C20:4n6)	0.01	0.01	0.01
19. cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.21	0.02	0.03
20. Heneicosanoic acid (C21:0)	ND	0.02	ND
21. Behenic acid (C22:0)	0.57	ND	0.05
22. Tricrosanoic acid (C23:0)	0.02	0.03	ND
SFA	15.89	10.82	8.63
MUFA	64.18	12.33	14.02
PUFA	19.92	76.85	77.35
UFA	84.11	89.18	91.37
Trans fat	0.04	0.03	0.03
Omega 3 (mg/100 g)	1,582.77	58,666.97	37,240.2
Omega 6 (mg/100 g)	18,307.79	18,141.29	40,061.54
Omega 9 (mg/100 g)	51,564.54	11,820.59	13,874.4
Omega 6:3 ratio	11.57	0.31	1.08

Note: ND = Not detected, Limit of detection = 0.01 g/100 g

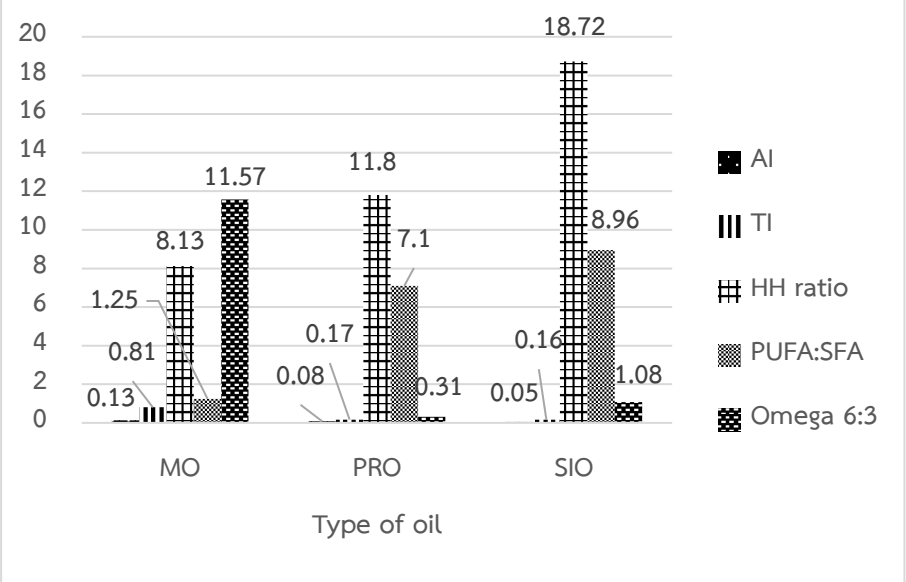


Figure 8 Nutritional properties of MO, PRO and SIO

Note: AI = Atherogenic index, TI= Thrombogenic index,
HH ratio = Hypocholesterolemic : hypercholesterolemic ratio

Results from the fatty acid composition were used to calculate for blending ratio of oils by aiming to get omega 6:3 ratio closest to 10:1. It was found that the suitable blending ratios (w/w) of MO:PRO were 0:100, 190:1, 200:1, 210:1, 220:1 and 100:0 and those for MO:SIO were 0:100, 100:1, 110:1, 120:1, 130:1, and 100:0. These ratio were further used in part 2.

Part 2 Blending oil for a balancing ratio of omega 6:3

For part 2, the selected 6 ratios of each MO:PRO and MO:SIO that had omega 6:3 closest to 10:1 were studied for their physical, chemical, and nutritional properties the same as in part 1. The results of blended oil properties were shown in Figures 9-13 and Tables 13-14.

Figures 9 and 10 showed physical properties of the blended oils. For color, decreasing amount of PRO or SIO used for blending with MO exhibited the lighter color of the blended oils as L* increased, less redness as a* decreased and less

yellowness as b* decreased. For viscosity, all the blended oils that contained 2 types of oil had no significant difference ($p>0.05$) in their viscosity. Moreover, decreasing either amount of PRO or SIO, the viscosity of the blended oil trended to increase. This phenomenon was contributed by the increased amount of MUFA in the blended oil, resulting in stacking together of the fatty acid molecules (Kim et al., 2010).

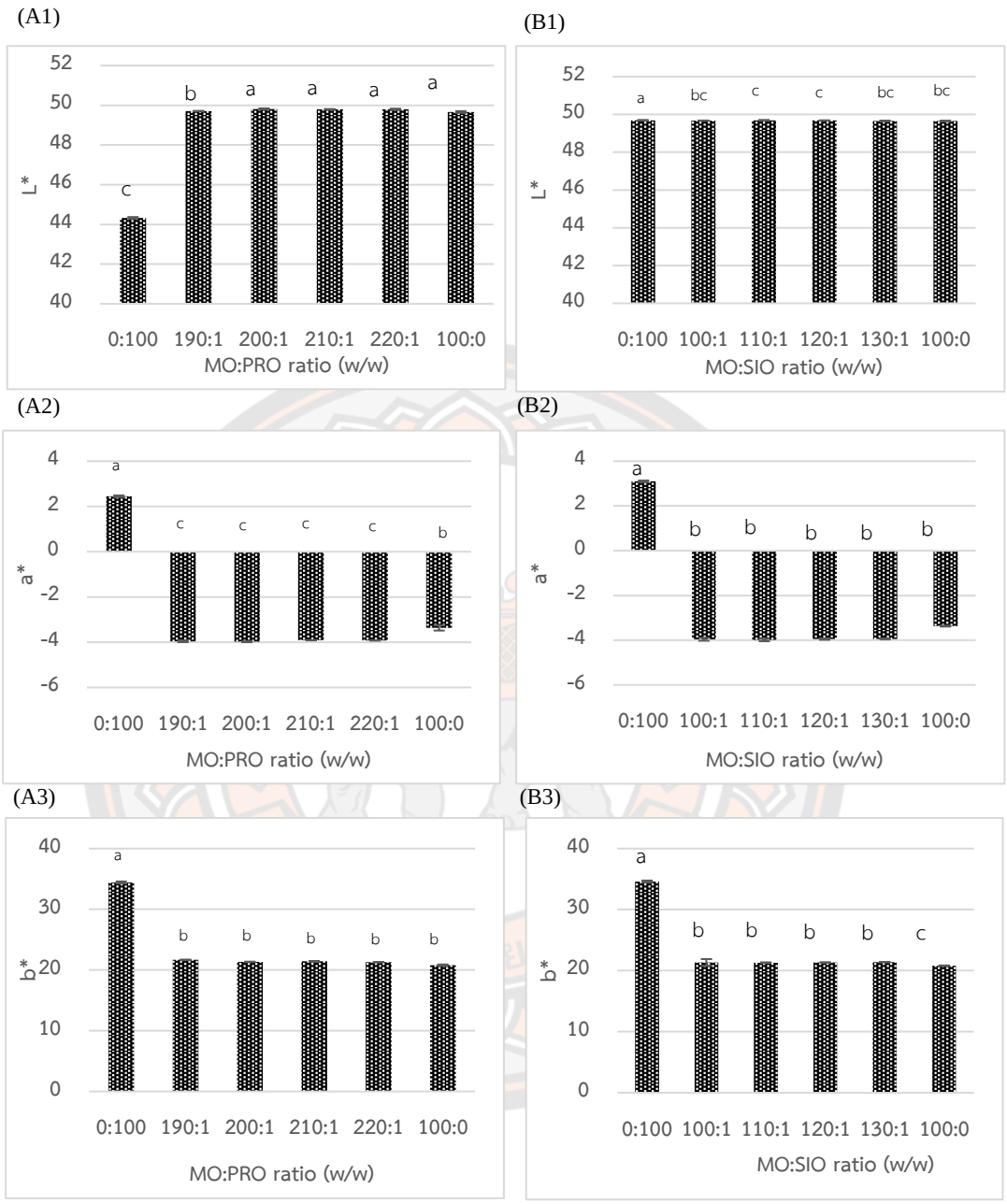


Figure 9 L* a* and b* of MO:PRO at different blending ratio (A1), (A2), (A3) respectively and L* a* and b* of MO:SIO at different blending ratio (B1), (B2), (B3), respectively

Note: Different letters (a-f) within a graph showed significantly different ($p<0.05$)

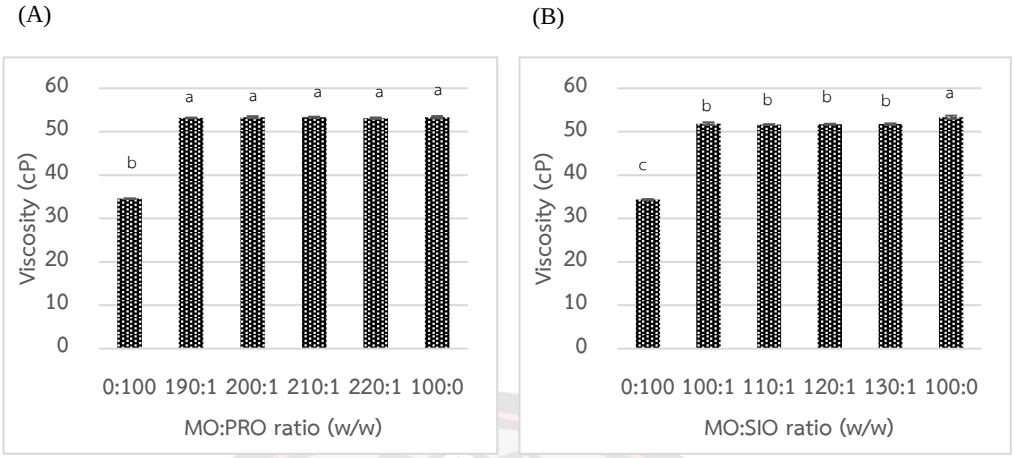
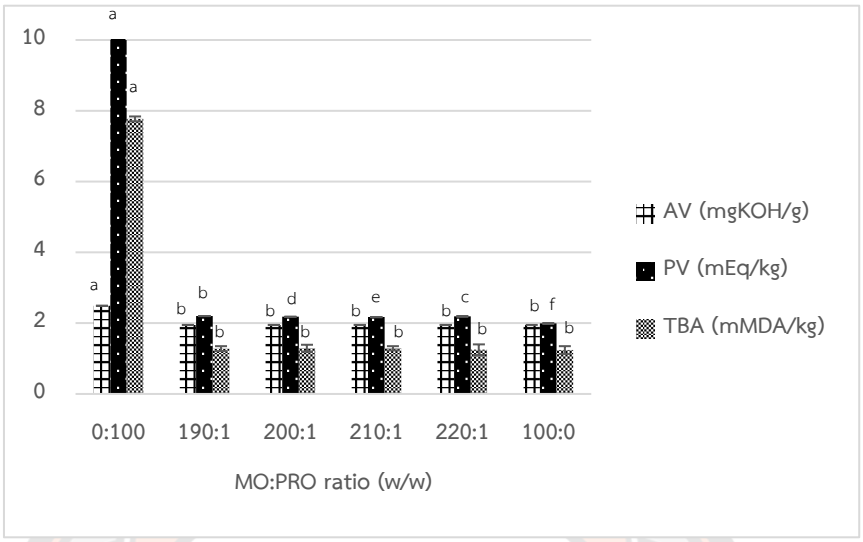


Figure 10 Viscosity of MO blened with PRO (A) and SIO (B)

Note: Different letters (a-c) within a graph showed significantly different ($p<0.05$)

Figures 11 and 12 showed chemical properties of the blended oils. Both blended oils exhibited similar results in that, blending either PRO or SIO to MO, the blended oil had lower AV, PV and TBA. Moreover, the blending ratio either MO:PRO or MO:SIO at 0:100 showed the highest PV and TBA. This indicated that this oil sample was oxidized greater than other ratios and was due to they contained more PUFA that could be easily oxidized. For antioxidant activity, both blended oils showed similar results in that, blending either PRO or SIO to MO, exhibited lower antioxidant activity for both DPPH and ABTS than parental PRO or SO. This finding was agreeable with the results of each parental oil presented in part 1.

(A)



(B)

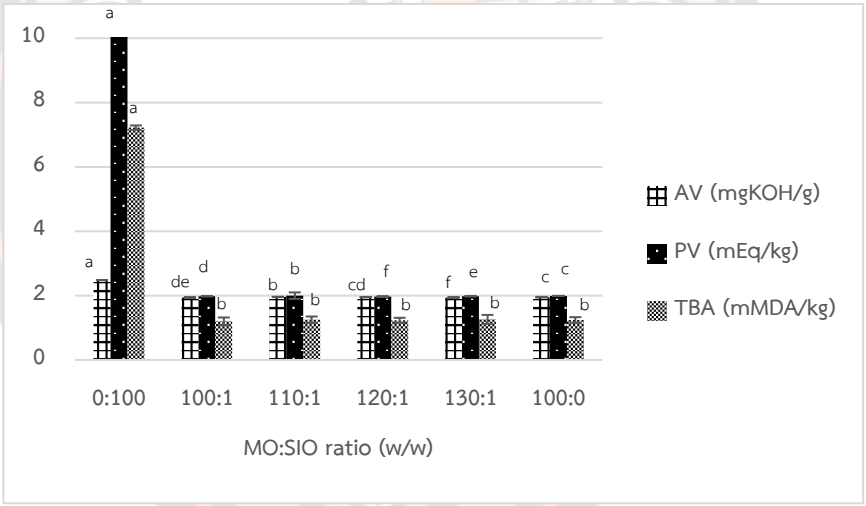
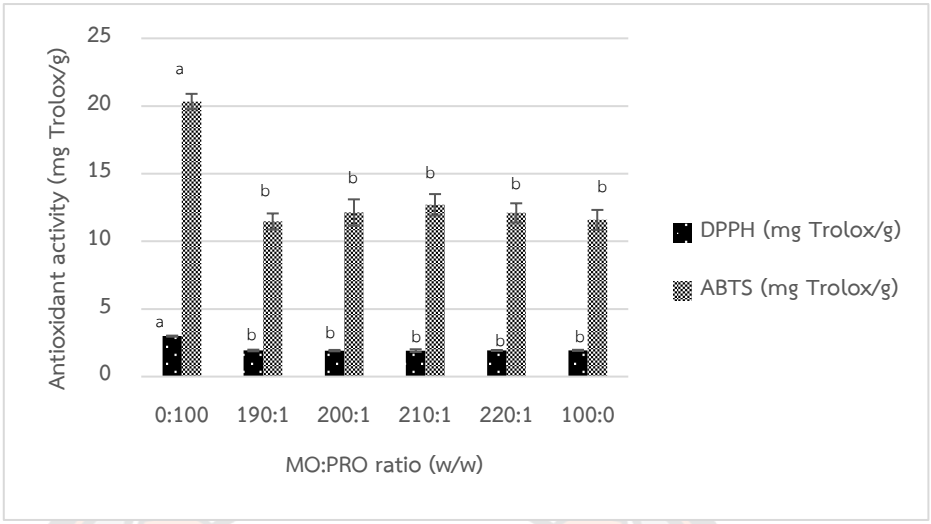


Figure 11 AV, PV and TBA of MO blended with PRO (A) and SIO (B)

Note: Different letters (a-c) within a same pattern bar in each graph showed significantly different ($p<0.05$)

(A)



(B)

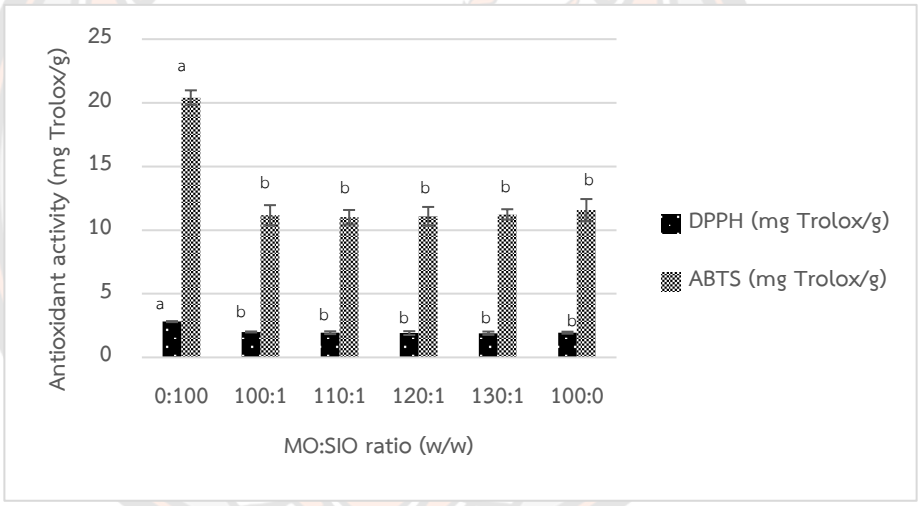


Figure 12 Antioxidant activity of MO blended with PRO (A) and SIO (B)

Note: Different letters (a-c) within a same pattern bar in each graph showed significantly different ($p<0.05$)

From the experiment, blending MO with PRO for 6 ratios and blending MO with SIO for another 6 ratios exhibited different fatty acid compositions of each blended oil. Fatty acid composition of MO:PRO and MO:SIO blended oils were shown in Tables 13 and 14, respectively. For fatty acid composition of MO blended either with PRO or SIO, 24 types of fatty acids were found consisting of 10 SFA, 6 MUFA, and 8 MUFA. Lower amount of PRO or SIO used for blending with MO contributed to higher MUFA and lower PUFA content in the blended oils since PUFA was the main fatty acid in PRO and SIO.

Table 13 Fatty acid composition (g/100 g of fat) of MO blended with PRO

Fatty acid	MO:PRO ratio (w/w)					
	0:100	**190:1**	**200:1**	**210:1**	**220:1**	**100:0**
1. Lauric acid (C12:0)	ND	0.06	0.05	0.07	0.06	0.06
2. Myristic acid (C14:0)	0.02	0.60	0.60	0.62	0.67	0.66
3. Palmitic acid (16:0)	7.48	8.56	8.56	8.87	8.48	8.1
4. Palmitoleic acid (C16:1n7)	0.22	9.73	9.13	9.90	10.82	11.11
5. Heptadecanoic acid (C17:0)	0.04	0.03	0.04	0.05	0.03	0.03
6. cis-10-Heptadecenoic acid (C17:1n10)	0.02	0.05	0.06	0.05	0.06	0.06
7. Stearic acid (C18:0)	3.02	4.40	4.40	4.47	4.34	4.35
8. trans-9-Elaidic acid (C18:1n9t)	0.03	0.05	0.05	0.07	0.05	0.04
9. cis-9-Oleic acid (C18:1n9c)	11.82	48.98	49.00	48.49	50.30	51.56
10. cis-9,12-Linoleic acid (C18:2n6)	18.06	18.80	19.80	20.24	21.15	18.27
11. gamma-Linolenic acid (C18:3n6)	0.04	ND	ND	ND	ND	ND
12. alpha-Linolenic acid (C18:3n3)	58.61	1.82	1.79	1.81	1.83	1.21
13. Arachidic acid (C20:0)	0.20	1.79	1.79	1.83	1.91	2.1
14. cis-11-Eicosenoic acid (C20:1n11)	0.24	1.18	1.18	1.20	1.31	1.41
15. cis-11,14-Eicosadienoic acid (C20:2)	0.04	0.03	0.03	0.10	0.03	0.03
16. cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.03	0.03	0.03	0.05	0.03	0.02
17. cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.03	ND	ND	ND	ND	0.16
18. Arachidonic acid (C20:4n6)	0.01	0.01	0.01	0.01	0.01	0.01
19. cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.02	0.07	0.07	0.13	0.06	0.21

Fatty acid	MO:PRO ratio (w/w)					
	0:100	**190:1**	**200:1**	**210:1**	**220:1**	**100:0**
20. Heneicosanoic acid (C21:0)	0.02	0.02	ND	ND	0.01	ND
21. Behenic acid (C22:0)	ND	0.47	0.47	0.51	0.50	0.57
22. Erucic acid (C22:1n9)	ND	0.13	0.12	0.13	0.14	ND
23. Tricosanoic acid (C23:0)	0.03	ND	ND	ND	ND	0.02
24. Lignoceric acid (C24:0)	ND	0.19	0.18	0.19	0.19	ND
SFA	10.82	16.12	16.10	16.61	16.20	15.89
MUFA	12.33	60.11	60.16	59.83	62.68	64.18
PUFA	76.85	23.77	23.73	23.57	21.12	19.92
UFA	89.18	83.88	83.90	83.39	83.80	84.11
Trans fat	0.03	0.05	0.05	0.07	0.05	0.04
Omega 3 (mg/100g)	58,666.97	1,889.07	1,855.30	1,945.62	1,888.33	1,582.77
Omega 6 (mg/100g)	18,141.29	18,853.06	19,849.81	20,524.30	21,202.43	18,307.79
Omega 9 (mg/100g)	11,820.59	49,102.08	49,127.42	48,614.53	50,443.61	51,564.54
Omega 6:3 ratio	0.31	9.98	10.70	10.55	11.23	11.567

Note: ND = Not detected, Limit of detection = 0.01 g/100 g

Table 14 Fatty acid composition (g/100 g of fat) of MO blended with SIO

Fatty acid	MO:SIO ratio (w/w)					
	0:100	**100:1**	**110:1**	**120:1**	**130:1**	**100:0**
1. Lauric acid (C12:0)	ND	0.07	0.06	0.06	0.06	0.06
2. Myristic acid (C14:0)	0.01	0.67	0.61	0.60	0.65	0.66
3. Palmitic acid (16:0)	4.85	8.43	8.51	8.48	8.43	8.1
4. Palmitoleic acid (C16:1n7)	0.05	10.82	9.82	9.79	10.55	11.11
5. Heptadecanoic acid (C17:0)	0.08	0.04	0.03	0.04	0.03	0.03
6. cis-10-Heptadecenoic acid (C17:1n10)	0.04	0.06	0.05	0.05	0.06	0.06
7. Stearic acid (C18:0)	3.46	4.32	4.39	4.39	4.33	4.35
8. trans-9-Elaidic acid (C18:1n9t)	0.03	0.05	0.05	0.05	0.05	0.04
9. cis-9-Oleic acid (C18:1n9c)	13.87	50.07	48.97	49.06	49.63	51.56
10. cis-9,12-Linoleic acid (C18:2n6)	39.87	19.43	20.77	21.70	22.92	18.27
11. gamma-Linolenic acid (C18:3n6)	0.18	ND	ND	0.01	0.01	ND
12. alpha-Linolenic acid (C18:3n3)	37.20	1.97	1.86	1.83	1.87	1.21
13. Arachidic acid (C20:0)	0.18	1.88	1.79	1.80	1.87	2.1
14. cis-11-Eicosenoic acid (C20:1n11)	0.03	1.23	1.16	1.16	1.09	1.41
15. cis-11,14-Eicosadienoic acid (C20:2)	0.05	0.03	0.03	0.03	0.03	0.03
16. cis-8,11,14-Eicosatrienoic acid (C20:3n6)	ND	0.03	0.03	0.03	0.03	0.02
17. cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.01	ND	ND	ND	ND	0.16
18. Arachidonic acid (C20:4n6)	0.01	0.01	0.01	0.01	0.01	0.01
19. cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.03	0.06	0.06	0.07	0.06	0.21
20. Heneicosanoic acid (C21:0)	ND	0.01	0.01	0.01	0.01	ND
21. Behenic acid (C22:0)	0.05	0.50	0.47	0.47	0.49	0.57
22. Erucic acid (C22:1n9)	ND	0.14	0.12	0.13	0.14	ND
23. Tricosanoic acid (C23:0)	ND	ND	ND	ND	ND	0.02
24. Lignoceric acid (C24:0)	ND	0.19	0.18	0.18	0.18	ND
SFA	8.63	16.10	16.05	16.04	16.06	15.89
MUFA	14.02	62.36	60.18	60.25	61.50	64.18
PUFA	77.35	21.54	23.77	23.71	22.44	19.92
UFA	91.37	83.90	83.95	83.96	83.94	84.11
Trans fat	0.03	0.05	0.05	0.05	0.05	0.04
Omega 3 (mg/100g)	37,240.20	2,036.62	1,926.35	1,909.24	1,937.28	1,582.77
Omega 6 (mg/100g)	40,061.54	19,475.42	20,818.77	21,771.70	22,970.03	18,307.79
Omega 9 (mg/100g)	13,874.40	50,209.82	49,094.38	49,190.04	49,760.92	51,564.54
Omega 6:3 ratio	1.08	9.56	10.81	11.40	11.86	11.57

Note: ND = Not detected, Limit of detection = 0.01 g/100 g

Most importantly, blending either PRO or SIO with MO was significantly reduced omega 6:3 ratio of MO, as expected. All blending ratio had omega 6:3 ratio close to 10. However, in this study, only one blending ratio that exhibited omega 6:3 closest to 10 was selected for each blended oil for further study. Blending MO:PRO at ratio of 190:1 or using PRO 0.52% (w/w) had omega 6:3 ratio equal to 9.98 and blending MO: SIO at ratio of 100:1 or using SIO 0.99% (w/w) had omega 6:3 ratio equal to 9.56. Therefore, these two blending ratio were selected for further developing to food supplement.

For the nutritional properties, the results were illustrated in Figure 13. It was found that blending PRO or SIO with MO at any ratio improved the nutritional properties of MO, as indicated by increasing in their HH and PUFA:SFA ratio and decreasing in their AI, TI, omega 6:3 ratio compared to the initial MO's nutritional properties. However, all the blended oils had the nutritional properties much lower than the initial PRO and SIO since they were used only tiny amount to blend with MO.

Data of L*, a*, b*, viscosity, AV, PV, TBA, antioxidant activity both DPPH and ABTS, omega 6 content, omega 3 content, AI, TI, HH ratio, PUFA:SFA, and omega 6:3 ratio were used for PCA analysis. It was found that all the values, except omega 6 content, classified the samples into 3 groups as shown in Figure 14. Group 1, the blue color represented pure MO, and the green color represented the blended oils of both MO:PRO and MO:SIO. Group 2, the red color indicated pure PRO. Finally group 3, the purple color indicated pure SIO. It was noted that pure MO was classified in the same group with the blended oils. This indicated that all the blended oils, both MO:PRO and MP:SIO, had the relationship of L*, a*, b*, viscosity, AV, PV, TBA, antioxidant activity both DPPH and ABTS, omega 3 content, AI, TI, HH ratio, PUFA:SFA, and omega 6:3 ratio with MO more than those of PRO and SIO. In other words, those values of PRO and SIO were clearly differentiate from a group of MO and the blended oils. It was agreeable with previous results that PRO and SIO had clearly differences in their physical, chemical and nutritional properties from those of MO and the blended oils. PC1 and PC2 of PCA analysis were able to describe the correlation of the samples at 99.79% for this experiment.

(A)

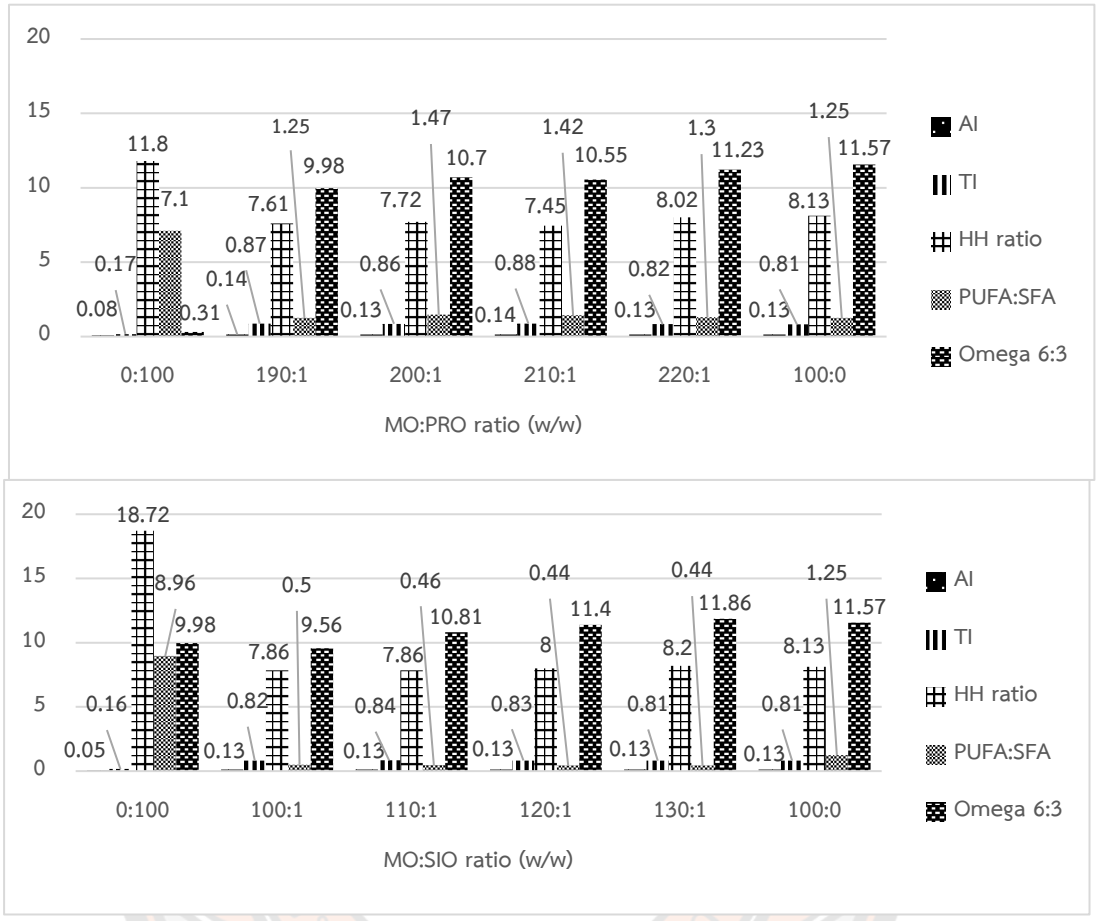


Figure 13 Nutritional properties of MO blended with PRO (A) and SIO (B)

Note: AI = Atherogenic index, TI = Thrombogenic index,
HH ratio = Hypocholesterolemic : hypercholesterolemic ratio

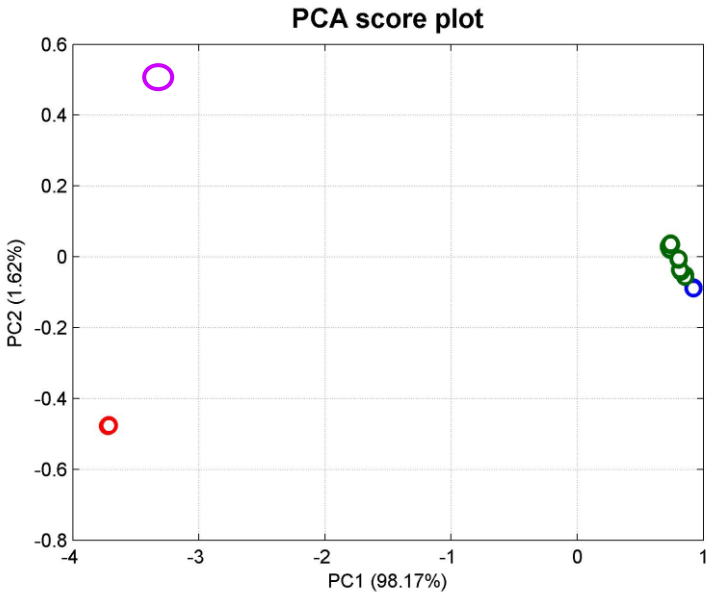


Figure 14 PCA plot of AV, PV, TBA, antioxidant activity both DPPH, and ABTS for MO, PRO, SIO and blended oils

Part 3 Capsule packing and shelf life study of the blended oil

For this experiment, the selected blending ratio of MO:PRO and MO: SIO was developed to food supplement. Blended oils (500 mg) were mechanically filled into hard capsules and 60 capsules of each formula were packed into three different types of packages including white plastic bottle, amber plastic bottle, and amber glass bottle (Figure 6). They were studied for changes in property at every 30 days for 120 days of storage at room temperature. Their properties including color, AV, PV, TBA and antioxidant activity both DPPH and ABTS were determined. The results were shown in Figures 15-18.

After storage for 120 days, some properties of the blended oil capsules for both MO:PRO and MO:SIO were significantly changed ($p<0.05$). With regard to storage time and type of package, after storage for 120 days, the color of both MO:PRO and MO:SIO capsules showed a slightly change changes as indicated by changes in L*, a*, and b* values. All the packages were able to prolong the change in color longer than 120 days.

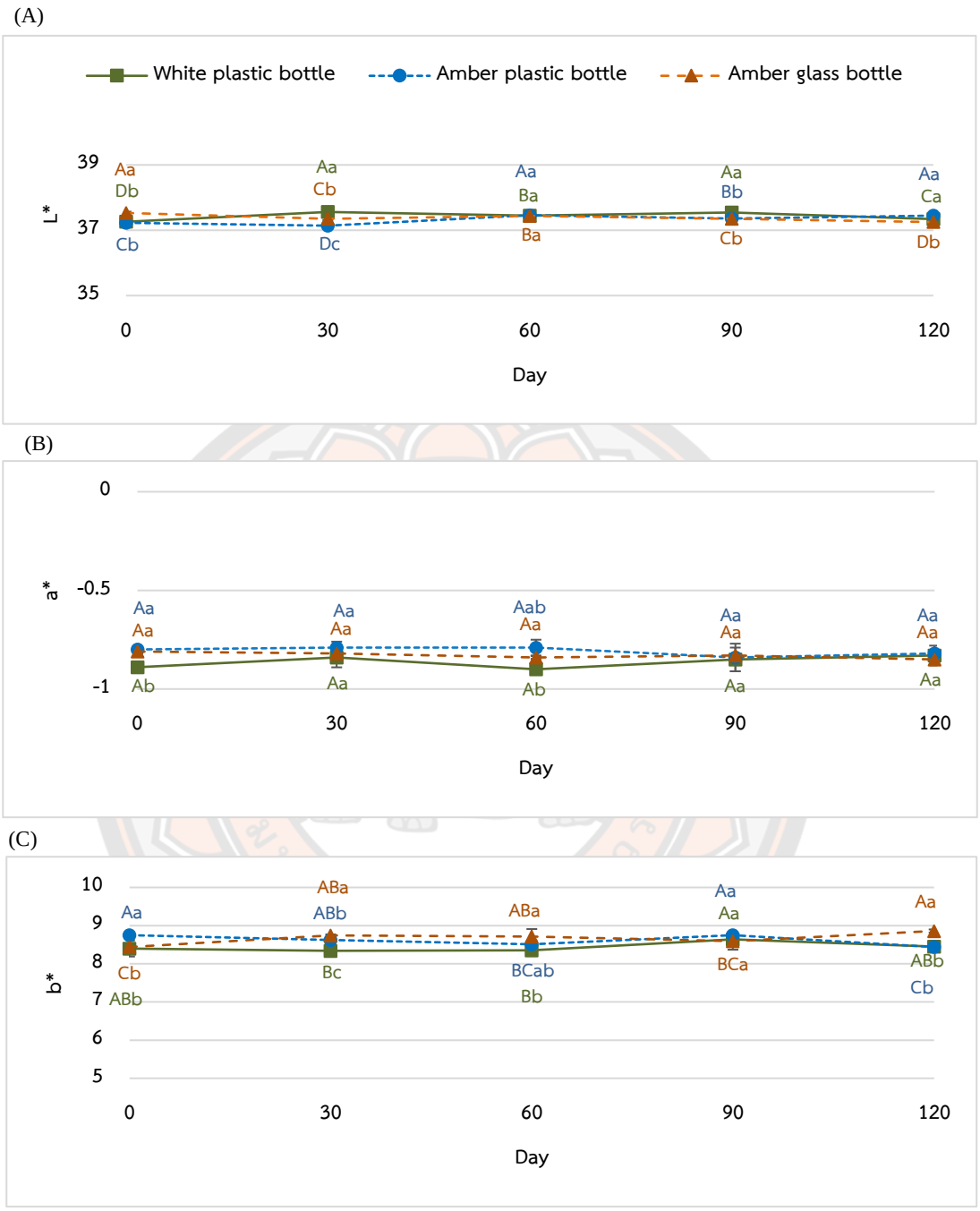


Figure 15 L* a* and b* of MO:PRO capsules storage in different packages (A), (B), (C) respectively

Note: Different small letters (a-c) indicated significant difference within same storage day ($p<0.05$)
Different capital letters (A-D) indicated significant difference within same type of package ($p<0.05$)

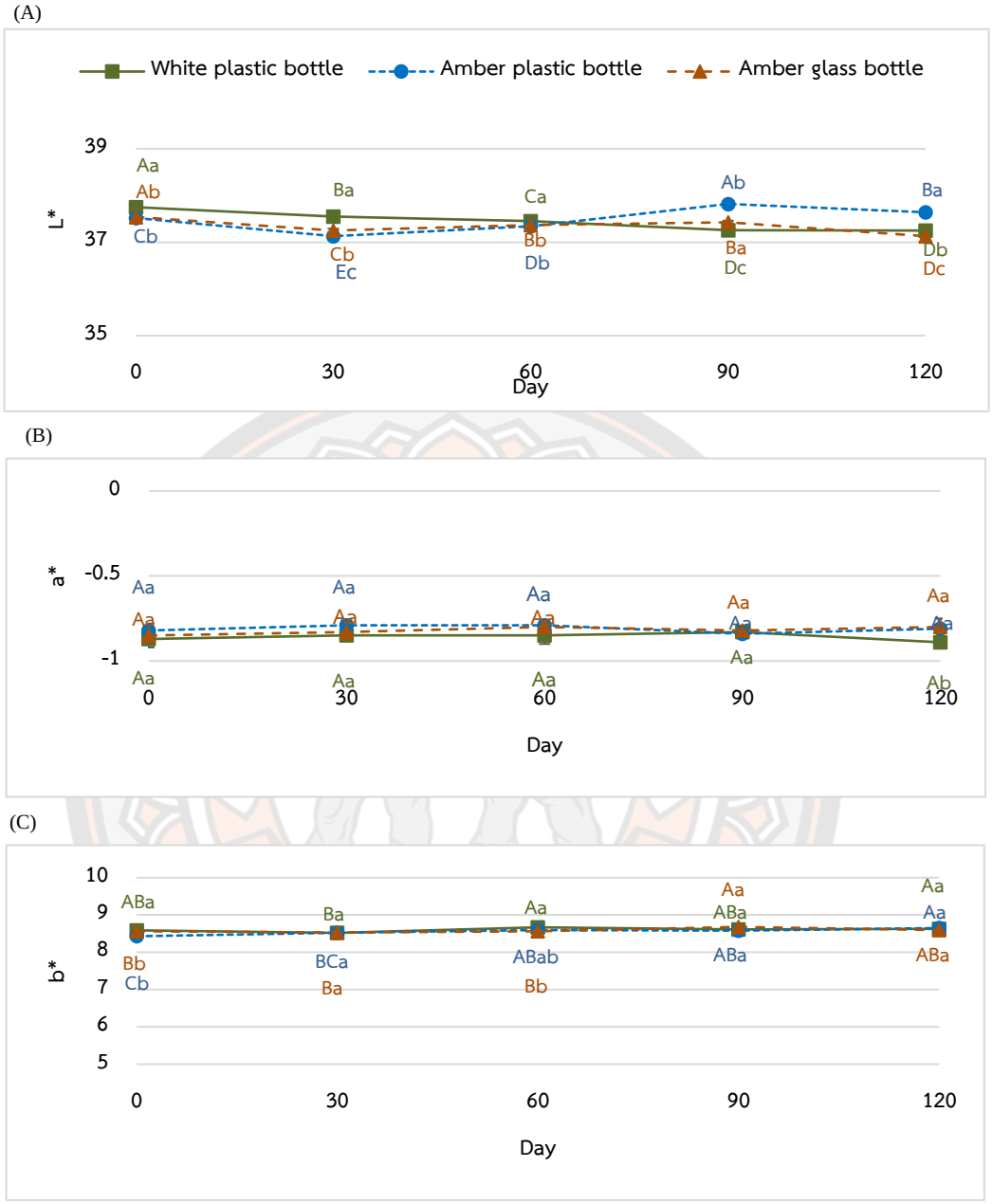


Figure 16 L* a* and b* of MO:SIO capsules storage in different packages (A), (B), (C) respectively

Note: Different small letters (a-c) indicated significant difference within same storage day ($p<0.05$)

Different capital letters (A-D) indicated significant difference within same type of package ($p<0.05$)

The results of chemical properties including AV, PV, TBA, and antioxidant activity both DPPH and ABTS were shown in Figures 17 and 18. Comparing storage time, it was clearly observed that PV and TBA value significantly increased during storage for both MO:PRO and MO:SIO capsules ($p<0.05$). However, these AV and PV were conformed to the standard values (4 mg KOH/g and 15 mEq/kg, respectively) after storage for 120 days for both MO:PRO and MO:SIO capsules. Furthermore, it was also found that antioxidant activity both DPPH and ABTS significantly decreased after storage ($p<0.05$). Comparing type of package, it was founded that amber glass bottle had more potential to prevent oxidation than the others due to it exhibited significantly lower PV and TBA value after storage for both MO:PRO and MO:SIO capsules ($p<0.05$). It meant that lower oxidative reaction occurred. This was contributed by a better oxygen barrier property of glass materials (Rababah et al., 2011), comparing to white plastic and amber plastic bottle that were made from PE and PET, respectively. As oxygen was one factor to catalyst the oxidative reaction, packages with greater oxygen barrier would have better prevention of oxidative reaction. Therefore, amber glass bottle was the most suitable package to prevent changes in properties of oil capsules during storage at room temperature.

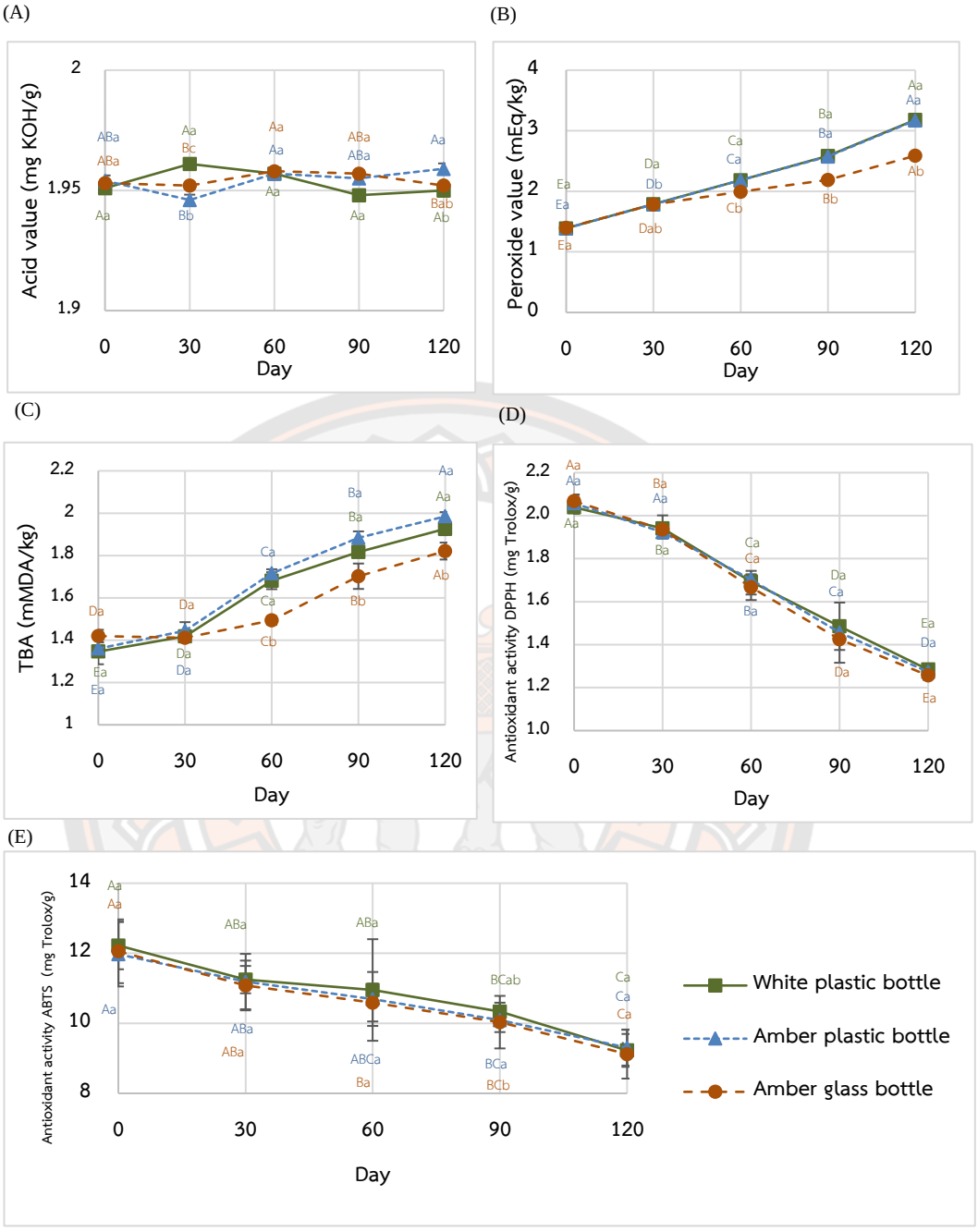


Figure 17 Acid value (A), Peroxide value (B), TBA value (C), Antioxidant activity DPPH (D), and ABTS (E) of MO:PRO capsules storage in different packages

Note: Different small letters (a-c) indicated significant difference within same storage day ($p<0.05$)

Different capital letters (A-D) indicated significant difference within same type of package ($p<0.05$)

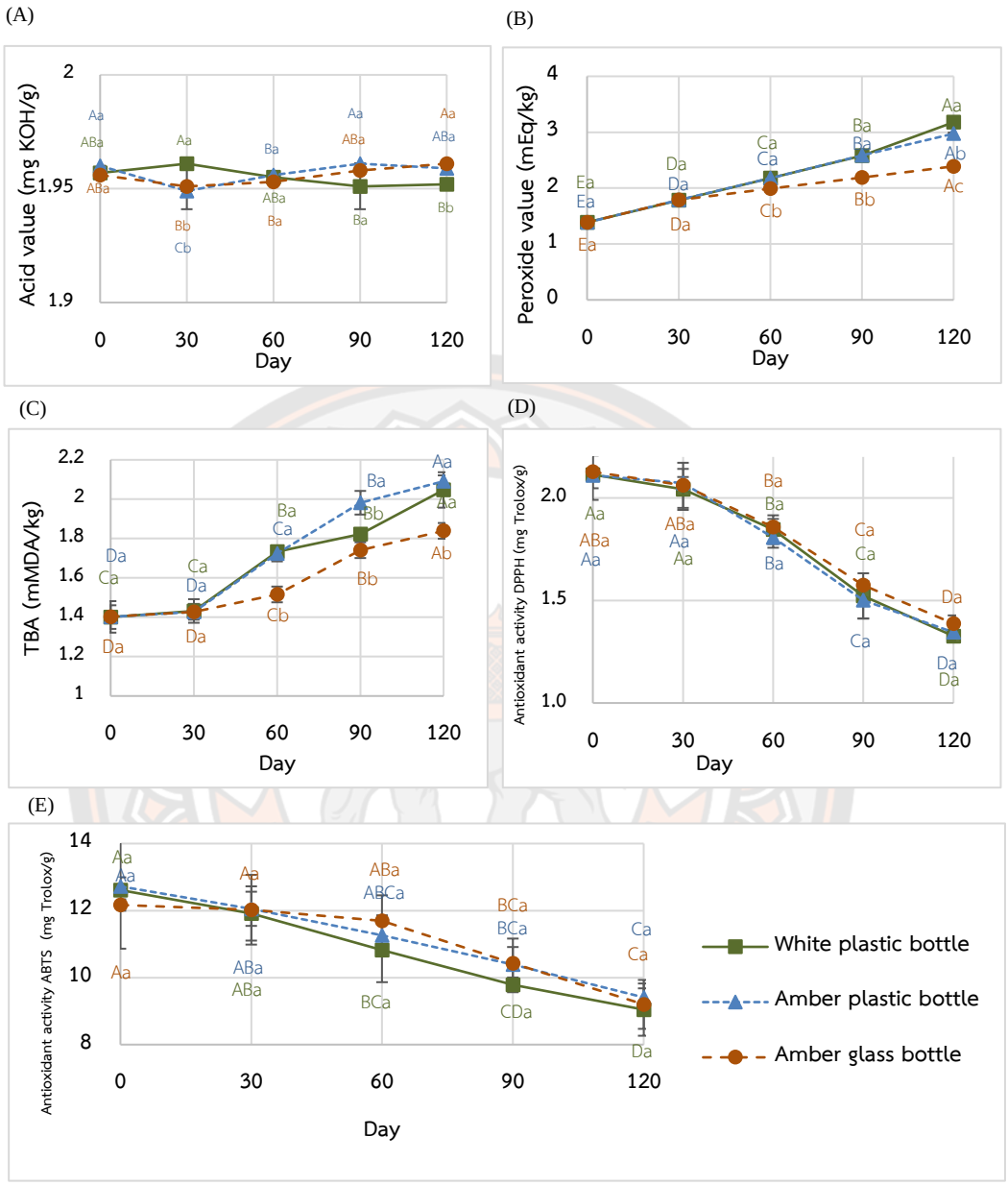


Figure 18 Acid value (A), Peroxide value (B), TBA value (C), Antioxidant activity DPPH (D), and ABTS (E) of MO:SIO capsules storage in different packages

Note: Different small letters (a-c) indicated significant difference within same storage day ($p<0.05$)

Different capital letters (A-D) indicated significant difference within same type of package ($p<0.05$)

Part 4 Production cost calculation of blended oil in capsules

The total production cost of MO:PRO and MO:SIO capsules packed in amber glass bottles was calculated by following equation and the results were shown in Tables 15.

$$\text{Total cost} = \frac{\text{(Raw materials cost + Labor cost + Production cost)}}{\text{Amount of product (Capsule)}}$$

Table 15 Raw materials and production cost of MO:PRO and MO: SIO capsules per bottle

Factor	Unit x cost per unit	Cost (Baht)	
		MO:PRO (190:1)	**MO:SIO (100:1)**
Raw materials			
Macadamia oil	1 kg x 1,600 Baht/kg	1,600	1,600
Perilla oil	0.005 kg x 660 Baht/kg	3.3	-
Sacha inchi oil	0.099 kg x 800 Baht/kg	-	79.2
Capsule	2,000 capsules x 0.12 Baht/capsule	240	240
Labor			
Worker	1 person	300	300
Production cost			
Oil extraction fee			
Macadamia kernels	Raw materials 2 kg x 20 Baht/kg	40	40
Perilla seeds	Raw materials 0.017 kg x 20 Baht/kg	0.34	-
Sacha inchi NIS	Raw materials 0.990 kg x 20 Baht/kg	-	19.80
Capsule filling fee	2,000 capsules x 0.40 Baht/capsule	800	800
Cost per 2,000 capsules		2,983.64	3,079.00
Cost per 1 capsule		1.49	1.54
Cost per 1 bottle (60 capsules)		89.40	92.40
Packaging cost (amber glass bottle)		16.60	16.60
Total cost per 1 bottle		**106.00**	**109.00**

The MO:PRO and MO: SIO capsules costed 1.49 and 1.54 Baht per capsule or 89.40 and 92.40 Baht/bottle, (60 capsules each), respectively. The total cost of MO:PRO and MO:SIO packed in amber glass bottle were 106 and 109 Baht per bottle, respectively. The production cost of MO:PRO and MO:SIO were different due to differences in raw materials cost, yield of oil extraction, and initial omega 6 and omega 3 content. The cost of MO:SIO was more expensive than MO:PRO due to SIO contained lower initial omega 3 content and lower extraction yield than PRO so higher amount of SIO was needed for balancing omega 6:3 ratio in MO. It must be noted that this production cost was calculated from the lab scale that raw materials and packages were purchased in small volume as well as, oil extraction and blended oil capsules filling were processed in a small batch. This cost at a pilot scale or commercial scale would be cheaper due to cheaper raw materials and packages. Moreover, as the oil had a balance ratio of omega 6:3, it was able to add value to the products and sell at a higher price than some available blended oil products. It could be sold as high price as up to 500 Baht per bottle. This could make a benefit of 394 and 391 Baht per bottle for MO:PRO and MO: SIO, respectively.

As indicated above, the cost of production of both blended oils were very similar. This indicated that macadamia producers could select either perilla oil or sacha inchi oil for blending with macadamia oil in order to improve its omega 6:3 ratio. However, perilla seeds are commonly more available in the Thai markets than sacha inchi NIS. This availability of the raw material could be taken into account for the selection.

Full kernels of macadamia might be able to sell at higher price than these blended oil capsules. However, blended oil supplements would be able and suitable for those broken and undersized macadamia kernels that normally sold at half price of the full kernels. So they would be added more value and sold in higher price. In addition, to be more competitive to other food supplement products in the market, other bioactive substances, particularly natural occurring ones could be added to these blended oils for providing greater health benefits. As well as, more attractive and creative packaging design could help to attract consumer’s attention.

Furthermore, all data in this research would be beneficial to macadamia industry, for further product development, not only for oil products. Especially the fatty acid composition results help to indicate the quality and health benefits of macadamia kernel. Higher MUFA content in macadamia kernels could be a selling point with greater price.

CHAPTER V

CONCLUSIONS

This chapter consisted of conclusion of the results and recommendations for improving of this research and for further study.

Conclusion

Macadamia oil (MO) from cold pressing process had light greenish yellow color while perilla oil (PRO) and sacha inchi oil (SIO) had reddish yellow color. SFA was found in low amount ranging from 8.63-15.89 g/100 g in three parental oils. MUFA was predominant in MO (64.18 g/100 g) while PUFA was mainly presented in both PRO (76.85 g/100 g) and SIO (77.35 g/100 g). This fatty acid composition was the major factor caused three parental oils different in their properties. MO had higher viscosity but lower acid value, peroxide value, TBA value, antioxidant activity both DPPH and ABTS, and nutritional properties than that of PRO and SIO.

Ratio of omega 6:3 of MO was 11.57, imbalance and higher than the recommended value by WHO, 4-10:1. PRO and SIO had lower omega 6:3 than MO, showing potentials to be used for blending with MO to reduce omega 6:3 ratio of MO. Fatty acid composition of the three parental oils was used to calculate for blending ratio of omega 6:3 to be close to 10:1. Successfully, it was exhibited final ratio of omega 6:3 of the blended oils close to 10:1, as expected. The blending ratio of MO:PRO and MO:SIO at 190:1 and 100:1 contributed the blended oils with omega 6:3 ratio of 9.98 and 9.56, respectively, that were closed to 10. It could be concluded that fatty acid composition could be used as a rough prediction for the final fatty acid content of the blended oil at any ratio.

The properties of the blended oil capsules were significantly changed after storage at room temperature for 120 days ($p<0.05$). Different types of packages also caused significant differences in oil properties after storage. However, properties of both blended oils, MO:PRO and MO:SIO, were still conformed to the standard after the storage period. It indicated that all packages, including white plastic, amber

plastic, and amber glass had potential to protect the physical and chemical properties of the oil longer than 120 days of storage. However, amber glass bottle had more potential to prevent chemical properties of oil capsules than white plastic and amber plastic bottle.

Comparing the used of PRO and SIO to blend with MO, they were not different in term of final properties and changes during storage of the blended oils since they had similar fatty acid compositions. However, they were different in term of cost of production due to PRO and SIO had different costs of raw materials, oil extraction yield and initial omega 6 and omega 3 contents. Therefore, the amount needed for blending with MO was different.

The production cost of MO:PRO capsules was cheaper than that of MO:SIO, 1.49 Baht/capsule or 106 Baht/bottle for MO:PRO, and 1.54 Baht/capsule or 109 Baht/ bottle for MO:SIO.

Recommendations

1. For shelf life study, a longer period of time was recommended or an accelerated shelf life study should be conducted in order to obtain the shelf life of the blended oil capsules in different packages.

2. The ratio of omega 6:3 ranging from 4-10 of the blended oils should be investigated due to comparing their properties.

3. The different raw materials with different harvest seasons should be studied to compare the fatty acid composition.

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APPENDIX

APPENDIX A L*, a* AND b* OF MO, PRO AND SIO FOR FIGURE 7

Sample	Color		
	L*	a*	b*
MO	49.70±0.01[a]	-3.24±0.01[c]	22.95±0.01[c]
PRO	45.82±0.02[c]	2.57±0.04[b]	35.74±0.11[a]
SIO	48.98±0.01[b]	3.06±0.04[a]	35.56±0.04[b]

Note: Means with different letter (a-c) within the same column and experiment are significantly different ($p<0.05$)

APPENDIX B PHYSICAL PROPERTIES OF MO:PRO AND MO: SIO FOR FIGURES 9 AND 10

Sample	Blending ratio	Color			Viscosity (cP)
		L*	a*	b*	
MO:PRO	0:100	44.33±0.05^{c}	2.45±0.12^{a}	34.42±0.11^{a}	34.56±0.27^{b}
	190:1	49.71±0.01^{b}	-3.96±0.04^{c}	21.67±0.05^{b}	53.16±0.09^{a}
	200:1	49.82±0.03^{a}	-3.98±0.01^{c}	21.34±0.04^{b}	53.34±0.24^{a}
	210:1	49.80±0.03^{a}	-3.91±0.01^{c}	21.42±0.05^{b}	53.36±0.10^{a}
	220:1	49.80±0.03^{a}	-3.92±0.01^{c}	21.29±0.02^{b}	53.11±0.16^{a}
	100:0	49.66±0.04^{b}	-3.37±0.04^{b}	20.81±0.16^{b}	53.33±0.09^{a}
MO:SIO	0:100	49.69±0.02^{a}	3.10±0.02^{a}	34.60±0.05^{a}	34.37±0.34^{c}
	100:1	49.67±0.02^{a}	-3.96±0.06^{b}	21.36±0.54^{b}	51.58±0.30^{b}
	110:1	49.69±0.01^{a}	-3.99±0.06^{b}	21.32±0.05^{b}	51.64±0.09^{b}
	120:1	49.68±0.01^{a}	-3.95±0.03^{b}	21.34±0.04^{b}	51.74±0.09^{b}
	130:1	49.65±0.01^{a}	-3.94±0.02^{b}	21.40±0.01^{b}	51.75±0.17^{b}
	100:0	49.65±0.04^{a}	-3.37±0.04^{b}	20.81±0.16^{c}	53.33±0.09^{a}

Note: Means with different letter (a-e) within the same column and experiment are significantly different ($p<0.05$)

APPENDIX C CHEMICAL PROPERTIES OF MO:PRO AND MO:SIO FOR FIGURES 11 AND 12

Sample	Blending ratio	AV (mg KOH/g)	PV (mEq/kg)	TBA (mMDA/kg)	Antioxidant activity (mg Trolox/g)	
					DPPH	ABTS
MO:PRO	0:100	2.49±0.00^{a}	10.96±0.01^{a}	7.77±0.11^{a}	2.99±0.04^{a}	20.33±0.74^{a}
	190:1	1.95±0.00^{b}	2.20±0.00^{c}	1.28±0.07^{b}	1.93±0.06^{b}	11.47±0.59^{b}
	200:1	1.95±0.00^{b}	2.18±0.00^{d}	1.29±0.10^{b}	1.92±0.05^{b}	12.13±0.97^{b}
	210:1	1.95±0.00^{b}	2.17±0.00^{e}	1.29±0.06^{b}	1.92±0.10^{b}	12.70±0.79^{b}
	220:1	1.95±0.00^{b}	2.19±0.00^{c}	1.25±0.15^{b}	1.93±0.01^{b}	12.10±0.71^{b}
	100:0	1.95±0.00^{b}	1.99±0.00^{f}	1.24±0.07^{b}	1.94±0.04^{b}	11.58±0.57^{b}
MO:SIO	0:100	2.48±0.01^{a}	10.59±0.00^{a}	7.22±0.09^{a}	2.81±0.08^{a}	20.41±0.86^{a}
	100:1	1.94±0.00^{de}	1.98±0.00^{d}	1.20±0.12^{b}	2.00±0.04^{b}	11.17±0.79^{b}
	110:1	1.96±0.00^{b}	2.00±0.01^{b}	1.25±0.10^{b}	1.94±0.11^{b}	11.01±0.57^{b}
	120:1	1.95±0.00^{cd}	1.97±0.00^{f}	1.23±0.08^{b}	1.91±0.15^{b}	11.09±0.73^{b}
	130:1	1.94±0.00^{f}	1.98±0.00^{e}	1.26±0.14^{b}	1.90±0.13^{b}	11.23±0.41^{b}
	100:0	1.95±0.00^{c}	1.99±0.00^{c}	1.24±0.04^{b}	1.94±0.04^{b}	11.58±0.57^{b}

Note: Means with different letter (a-f) within the same column, same blended oil and same experiment are significantly different ($p<0.05$)

APPENDIX D L* a* AND b* OF MO:PRO CAPSULES STORAGE IN DIFFERENT PACKAGES FOR FIGURE 15

Type of package	Time of storage (day)	Color		
		L*	a*	b*
White plastic bottle	0	37.26 ± 0.03^{Ab}	-0.89 ± 0.02^{Ab}	8.40 ± 0.21^{ABb}
	30	37.56 ± 0.02^{Aa}	-0.84 ± 0.05^{Aa}	8.34 ± 0.05^{Bc}
	60	37.45 ± 0.04^{Ba}	-0.90 ± 0.01^{Ab}	8.35 ± 0.04^{Bb}
	90	37.54 ± 0.04^{Aa}	-0.85 ± 0.06^{Aa}	8.64 ± 0.18^{Aa}
	120	37.34 ± 0.04^{Ca}	-0.83 ± 0.05^{Aa}	8.45 ± 0.04^{ABb}
Amber plastic bottle	0	37.23 ± 0.03^{Cd}	-0.80 ± 0.01^{Aa}	8.75 ± 0.05^{Aa}
	30	37.14 ± 0.04^{Dc}	-0.79 ± 0.03^{Aa}	8.62 ± 0.07^{ABb}
	60	37.46 ± 0.04^{Aa}	-0.84 ± 0.04^{Aab}	8.51 ± 0.10^{BCab}
	90	37.36 ± 0.04^{Bb}	-0.80 ± 0.07^{Aa}	8.75 ± 0.05^{Aa}
	120	37.42 ± 0.03^{Aa}	-0.82 ± 0.01^{Aa}	8.44 ± 0.04^{Cb}
Amber glass bottle	0	37.53 ± 0.04^{Aa}	-0.81 ± 0.03^{Aa}	8.44 ± 0.04^{Cb}
	30	37.35 ± 0.04^{Cb}	-0.82 ± 0.04^{Aa}	8.74 ± 0.03^{ABa}
	60	37.44 ± 0.03^{Ba}	-0.84 ± 0.02^{Aa}	8.71 ± 0.20^{ABa}
	90	37.35 ± 0.04^{Cb}	-0.83 ± 0.01^{Aa}	8.59 ± 0.22^{BCa}
	120	37.25 ± 0.04^{Db}	-0.85 ± 0.03^{Aa}	8.86 ± 0.04^{Aa}

Note: Means with different small letter (a-c) within the same column, and same experiment indicated significant difference within same storage day ($p<0.05$)

Means with different capital letter (a-c) within the same column, and same experiment indicated significant difference within same type of package ($p<0.05$)

APPENDIX E L* a* AND b* OF MO: SIO CAPSULES STORAGE IN DIFFERENT PACKAGES FOR FIGURE 16

Type of package	Time of storage (day)	Color		
		L*	a*	b*
White plastic bottle	0	37.75 ± 0.04^{Aa}	-0.87 ± 0.05^{Aa}	8.59 ± 0.02^{ABa}
	30	37.55 ± 0.04^{Ba}	-0.85 ± 0.03^{Aa}	8.52 ± 0.04^{Ba}
	60	37.45 ± 0.04^{Ca}	-0.85 ± 0.05^{Aa}	8.67 ± 0.03^{Aa}
	90	37.26 ± 0.03^{Dc}	-0.83 ± 0.03^{Aa}	8.61 ± 0.07^{ABa}
	120	37.25 ± 0.03^{Db}	-0.89 ± 0.01^{Ab}	8.63 ± 0.06^{Aa}
Amber plastic bottle	0	37.52 ± 0.02^{Cb}	-0.82 ± 0.04^{Aa}	8.43 ± 0.06^{Cb}
	30	37.13 ± 0.02^{Ec}	-0.79 ± 0.03^{Aa}	8.52 ± 0.05^{BCa}
	60	37.34 ± 0.05^{Db}	-0.79 ± 0.03^{Aa}	8.60 ± 0.03^{ABab}
	90	37.82 ± 0.02^{Ab}	-0.84 ± 0.02^{Aa}	8.58 ± 0.05^{ABa}
	120	37.64 ± 0.02^{Ba}	-0.81 ± 0.06^{Aa}	8.65 ± 0.04^{Aa}
Amber glass bottle	0	37.54 ± 0.05^{Ab}	-0.85 ± 0.01^{Aa}	8.56 ± 0.06^{Bb}
	30	37.25 ± 0.04^{Cb}	-0.83 ± 0.06^{Aa}	8.53 ± 0.05^{Ba}
	60	37.37 ± 0.03^{Bb}	-0.80 ± 0.07^{Aa}	8.56 ± 0.05^{Bb}
	90	37.43 ± 0.03^{Ba}	-0.82 ± 0.03^{Aa}	8.68 ± 0.04^{Aa}
	120	37.13 ± 0.03^{Dc}	-0.80 ± 0.01^{Aa}	8.60 ± 0.04^{ABa}

Note: Means with different small letter (a-c) within the same column, and same experiment indicated significant difference within same storage day ($p<0.05$)

Means with different capital letter (a-c) within the same column, and same experiment indicated significant difference within same type of package ($p<0.05$)

APPENDIX F CHEMICAL PROPERTIES OF MO:PRO CAPSULES STORAGE IN DIFFERENT PACKAGES FOR FIGURE 17

Type of package	Time of storage (day)	AV (mg KOH/g)	PV (mEq/kg)	TBA (mMDA/kg)	Antioxidant activity (mg Trolox/g)	
					DPPH	ABTS
White plastic bottle	0	1.951 ± 0.01^{Aa}	1.390 ± 0.00^{Ea}	1.346 ± 0.05^{Ea}	2.039 ± 0.03^{Aa}	12.222 ± 0.68^{Aa}
	30	1.961 ± 0.00^{Aa}	1.790 ± 0.01^{Da}	1.418 ± 0.02^{Da}	1.941 ± 0.02^{Ba}	11.247 ± 0.39^{ABa}
	60	1.957 ± 0.01^{Aa}	2.185 ± 0.01^{Ca}	1.681 ± 0.04^{Ca}	1.693 ± 0.06^{Ca}	10.951 ± 1.45^{ABa}
	90	1.948 ± 0.01^{Aa}	2.587 ± 0.01^{Ba}	1.816 ± 0.02^{Ba}	1.485 ± 0.05^{Da}	10.339 ± 0.25^{BCab}
	120	1.950 ± 0.01^{Ab}	3.180 ± 0.01^{Aa}	1.926 ± 0.03^{Aa}	1.283 ± 0.02^{Ea}	9.227 ± 0.47^{Ca}
Amber plastic bottle	0	1.954 ± 0.01^{ABa}	1.389 ± 0.01^{Ea}	1.360 ± 0.03^{Ea}	2.057 ± 0.04^{Aa}	11.972 ± 0.92^{Aa}
	30	1.946 ± 0.00^{Bc}	1.782 ± 0.00^{Db}	1.445 ± 0.04^{Da}	1.923 ± 0.03^{Aa}	11.192 ± 0.79^{ABa}
	60	1.957 ± 0.01^{Aa}	2.183 ± 0.01^{Ca}	1.715 ± 0.02^{Ca}	1.702 ± 0.02^{Ba}	10.695 ± 0.77^{ABCa}
	90	1.955 ± 0.01^{ABa}	2.574 ± 0.00^{Ba}	1.884 ± 0.03^{Ba}	1.456 ± 0.14^{Ca}	10.089 ± 0.34^{BCa}
	120	1.959 ± 0.00^{Aa}	3.172 ± 0.02^{Aa}	1.985 ± 0.02^{Aa}	1.273 ± 0.02^{Da}	9.305 ± 0.51^{Ca}
Amber glass bottle	0	1.953 ± 0.00^{ABa}	1.397 ± 0.00^{Ea}	1.419 ± 0.03^{Da}	2.068 ± 0.03^{Aa}	12.056 ± 0.91^{Aa}
	30	1.952 ± 0.00^{Bb}	1.786 ± 0.01^{Dab}	1.411 ± 0.01^{Da}	1.935 ± 0.02^{Ba}	11.081 ± 0.71^{ABa}
	60	1.958 ± 0.00^{Aa}	1.994 ± 0.00^{Cb}	1.493 ± 0.02^{Cb}	1.667 ± 0.08^{Ca}	10.592 ± 0.54^{Ba}
	90	1.957 ± 0.01^{ABa}	2.190 ± 0.01^{Bb}	1.702 ± 0.06^{Bb}	1.425 ± 0.04^{Da}	10.033 ± 0.75^{Bcb}
	120	1.952 ± 0.00^{Bab}	2.588 ± 0.01^{Ab}	1.821 ± 0.04^{Ab}	1.256 ± 0.06^{Ea}	$9.1220.70^{Ca}$

Note: Means with different small letter (a-c) within the same column, and same experiment indicated significant difference within same storage day ($p<0.05$)

Means with different capital letter (a-c) within the same column, and same experiment indicated significant difference within same type of package ($p<0.05$)

APPENDIX G CHEMICAL PROPERTIES OF MO: SIO CAPSULES STORAGE IN DIFFERENT PACKAGES FOR FIGURE 18

Type of package	Time of storage (day)	AV (mg KOH/g)	PV (mEq/kg)	TBA (mMDA/kg)	Antioxidant activity (mg Trolox/g)	
					DPPH	ABTS
White plastic bottle	0	1.387 ± 0.01^{Ea}	1.401 ± 0.06^{Ca}	1.998 ± 0.02^{Aa}	12.611 ± 0.38^{Aa}	1.387 ± 0.01^{Ea}
	30	1.782 ± 0.00^{Da}	1.432 ± 0.06^{Ca}	2.042 ± 0.10^{Aa}	11.914 ± 0.81^{ABa}	1.782 ± 0.00^{Da}
	60	2.177 ± 0.00^{Ca}	1.733 ± 0.03^{Ba}	1.845 ± 0.07^{Ba}	10.823 ± 0.96^{BCa}	2.177 ± 0.00^{Ca}
	90	2.580 ± 0.01^{Ba}	1.822 ± 0.03^{Bb}	1.523 ± 0.11^{Ca}	9.783 ± 0.22^{CDa}	2.580 ± 0.01^{Ba}
	120	3.184 ± 0.01^{Aa}	2.047 ± 0.09^{Aa}	1.326 ± 0.03^{Da}	9.044 ± 0.78^{Da}	3.184 ± 0.01^{Aa}
Amber plastic bottle	0	1.385 ± 0.00^{Ea}	1.402 ± 0.08^{Da}	1.975 ± 0.12^{Aa}	12.722 ± 1.86^{Aa}	1.385 ± 0.00^{Ea}
	30	1.787 ± 0.01^{Da}	1.428 ± 0.04^{Da}	2.072 ± 0.03^{Aa}	12.053 ± 0.51^{ABa}	1.787 ± 0.01^{Da}
	60	2.179 ± 0.00^{Ca}	1.724 ± 0.04^{Ca}	1.807 ± 0.05^{Ba}	11.259 ± 0.60^{ABCa}	2.179 ± 0.00^{Ca}
	90	2.583 ± 0.01^{Ba}	1.982 ± 0.06^{Ba}	1.501 ± 0.09^{Ca}	10.394 ± 0.77^{BCa}	2.583 ± 0.01^{Ba}
	120	2.974 ± 0.02^{Ab}	2.091 ± 0.03^{Aa}	1.345 ± 0.04^{Da}	9.409 ± 0.27^{Ca}	2.974 ± 0.02^{Ab}
Amber glass bottle	0	1.385 ± 0.00^{Ea}	1.403 ± 0.01^{Da}	1.990 ± 0.08^{ABa}	12.167 ± 0.17^{Aa}	1.385 ± 0.00^{Ea}
	30	1.789 ± 0.01^{Da}	1.428 ± 0.03^{Da}	2.061 ± 0.11^{ABa}	12.025 ± 1.04^{Aa}	1.789 ± 0.01^{Da}
	60	1.990 ± 0.00^{Cb}	1.516 ± 0.04^{Cb}	1.858 ± 0.04^{Ba}	11.695 ± 0.76^{ABa}	1.990 ± 0.00^{Cb}
	90	2.188 ± 0.00^{Bb}	1.741 ± 0.04^{Bb}	1.574 ± 0.02^{Ca}	10.422 ± 0.49^{BCa}	2.188 ± 0.00^{Bb}
	120	2.389 ± 0.01^{Ac}	1.839 ± 0.04^{Ab}	1.387 ± 0.04^{Da}	9.201 ± 0.73^{Ca}	2.389 ± 0.01^{Ac}

Note: Means with different small letter (a-c) within the same column, and same experiment indicated significant difference within same storage day ($p<0.05$)

Means with different capital letter (a-c) within the same column, and same experiment indicated significant difference within same type of package ($p<0.05$)

APPENDIX H CALCULATION OF PRODUCTION COST

The production cost of MO:PRO and MO:SIO capsules were calculated by following equation;

$$\text{Total cost (Baht)} = \frac{\text{(Raw materials cost) + (Labor cost) + (Production cost)}}{\text{Amount of product (Capsule)}}$$

Calculation of MO:PRO capsule

Raw materials = Macadamia oil + Perilla oil + Capsules
= 1,600 + 3.30 + 240 Baht
= 1,843.30 Baht

Labor cost = 1 Worker
= 300 Baht

Production cost = Macadamia oil extraction fee + Perilla oil extraction fee + Capsule filling fee
= 40 + 0.34 + 800 Baht
= 840.34 Baht

Amount of product = Amount of capsules
= 2,000 capsules

Substitute the values in the equation;

Total cost = (1,843.30 + 300 + 840.34 Baht) / (2,000 capsules)
= 1.49 Baht/capsule

To calculate cost/bottle (packed 60 capsules in amber glass bottle)

Total cost/bottle = (Cost per capsule x 60 capsules) + Packaging cost
= (1.49 Baht x 60 capsules) + 16.60 Baht
= 109 Baht/bottle

Calculation of MO: SIO capsule

Raw materials = Macadamia oil + Sacha inchi oil + Capsules
= 1,600 + 79.20 + 240 Baht
= 1,919.20 Baht

Labor cost = 1 Worker
= 300 Baht

Production cost = Macadamia oil extraction fee +
Sacha inchi oil extraction fee + Capsule filling fee
= 40 + 19.80 + 800 Baht
= 859.80 Baht

Amount of product = Amount of capsules
= 2,000 capsules

Substitute the values in the equation;

Total cost = (1,919.20 + 300 + 859.80 Baht) / (2,000 capsules)
= 1.54 Baht/capsule

To calculate cost/bottle (packed 60 capsules in amber glass bottle)

Total cost/bottle = (Cost per capsule x 60 capsules) + Packaging cost
= (1.54 Baht x 60 capsules) + 16.60 Baht
= 109 Baht/bottle

APPENDIX I MEASUREMENT METHOD

Color measurement

Color measurement was done by the Minolta model CR-100, USA colorimeter and the data were reported as L* (lightness), a* (greenness to redness), and b* (blueness to yellowness)

Viscosity measurement

The viscosity of the oil samples was measured by Brookfield Viscometer (RVDV ll, Model 845X, USA). It was a measuring the force required to rotate a spindle in a fluid. Sample of 15 mL was added in a container and probe No. 0 was used to detect the viscosity at 20 rpm, room temperature and the data were collected

Acid value (AV)

AV was measured by AOCS (2004) using the titration method. A mixture of 25 mL diethyl ether, 25 mL ethyl alcohol and 1 mL phenolphthalein 1% was prepared and titrated with sodium hydroxide 0.1 N until a light purple color appeared. Then, the mixture was added to 5 g of oil sample. The mixture was titrated with sodium hydroxide 0.1 N again until a purple color appeared. The volume of both titrations was read and recorded. Acid value was calculated using the formula below:

$$\text{Acid Value (mg KOH/g)} = \frac{\text{(Volume of NaOH used (mL) x Concentration of NaOH (N) x 56.1)}}{\text{Weight of sample (g)}}$$

Peroxide value (PV)

Oil sample was weighed 5 g and poured into a 250 mL Erlenmeyer flask. Acetic acid mixing with chloroform solution at a ratio of 3:2 (v/v) 30 mL was added and the flask was swirled until oil dissolved. To avoid inhalation of the vapors carcinogen from chloroform, this process was done in a fume hood. Saturated potassium iodide solution 0.5 mL was added in the flask and thoroughly mixed. The solution was maintained for 1 min with occasional shaking and then 30 mL of

distilled water was added and shaked vigorously to liberate the iodine from the chloroform layer.

The solution was titrated with 0.1 N sodium thiosulfate until the yellow color disappeared. One percentage of starch solution indicator 0.5 mL was added and shaked. The sodium thiosulfate was titrated again until the violet color appeared. PV was calculated according to the following equation:

$$PV\ (mEq/kg) = [(S - B) \times N \times 1000] / W$$

Where:

S = volume (mL) of sodium thiosulfate used to titrate the sample

B = volume (mL) of sodium thiosulfate used for the blank

N = normality of the standardized sodium thiosulfate solution

W = weight of the sample (g)

Thiobarbituric acid (TBA) value

Sample solution was prepared by mixing 1 g of oil with 4 ml 1-butanol in screw cap tube. TBA solution (1% in 1-butanol) 5 ml was added and mixed well. The solution was incubated in water bath at 95 °C for 2 h. After that the tube was removed and cooled by tap water until reach room temperature. The cooled solution was measured for absorbance at 532 nm. TBA value calculated comparing with standard curve of malondialdehyde and reported as mMDA/kg.

Antioxidant activity

DPPH assay

The sample solution was prepared by weighing 0.10 g of oil sample and dissolved with 1 mL of 95% ethanol. Sample solution 0.1 mL was mixed with 2 mL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol. A blank was prepared using the reaction solvent without sample. Absorbance at 517 nm was determined after leaving the sample and the blank for 30 min in the dark at room temperature. The absorbance was calculated to antioxidant activity by comparing with Trolox standard curve and reported as mg Trolox/g. Trolox standard curve was prepared at concentration of 0-80 ppm.

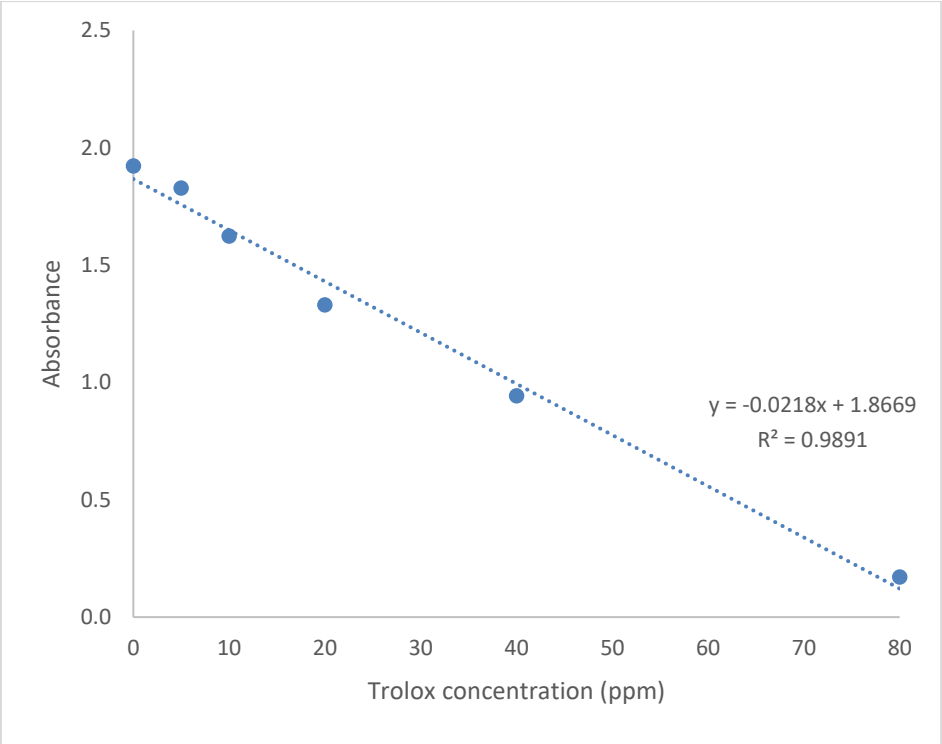

Figure 19 Trolox standard curve for DPPH assay

ABTS assay

The sample solution was prepared by weighing 0.1 g of oil sample and dissolved with 1 mL of 95% ethanol. ABTS stock solution was prepared by mixing 2.45 mM potassium persulphate solution and 7 mM ABTS solution in equal volume to react for 16 h in the dark at room temperature before use and diluted with ethanol until reaching absorbance of 0.7 at 734 nm. Then 0.02 mL of sample solution was mixed with 0.98 mL of ABTS stock solution. Absorbance at 734 nm was determined after leaving the sample and the blank for 30 min in the dark at room temperature. A blank was prepared using the reaction solvent without sample. The absorbance was calculated to antioxidant activity by comparing with Trolox standard curve and reported as mg Trolox/g. Trolox standard curve was prepared at concentration of 0-80 ppm.

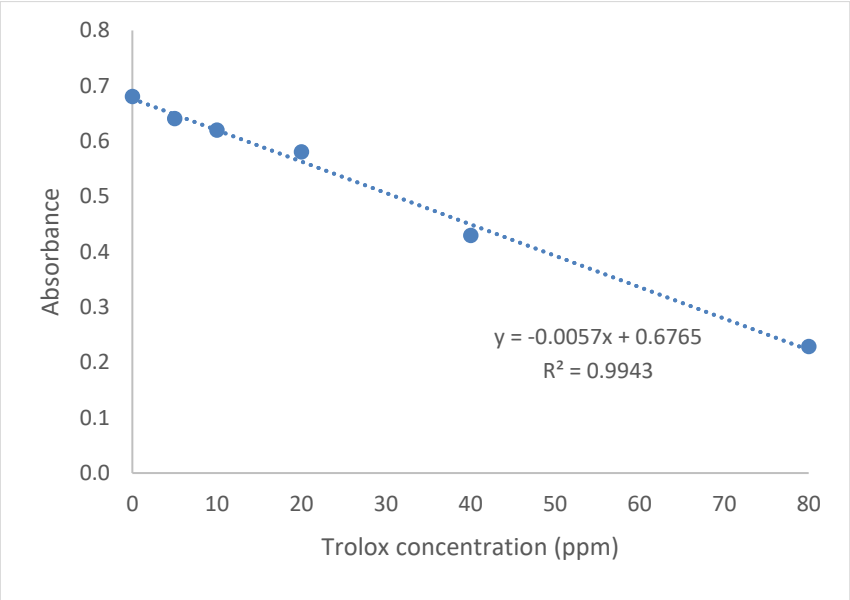

Figure 20 Trolox standard curve for ABTS assay

Nutritional properties

All the nutrition properties were calculated from the fatty acid composition data by the following equations:

Atherogenic index (AI) $= \frac{C12{:}0 + (4\,x\,C14{:}0) + C16{:}0}{\sum MUFA + \sum \omega 6 + \sum \omega 3}$

Thrombogenic index (TI) $= \frac{\mathrm{C14{:}0 + (4\,x\,C16{:}0)\,x\,C18{:}0}}{(0.5\,\mathrm{x}\,\sum \mathrm{MUFA}) + (0.5\,\mathrm{x}\,\sum \omega 6) + (3\,\mathrm{x}\,\sum \omega 3)}$

Hypocholesterolemic: hypercholesterolemic $(HH) ratio$

$$= \frac{C18{:}1 + C18{:}2 + C20{:}4 + C18{:}3 + C20{:}5 + C22{:}5 + C22{:}6}{C14{:}0 + C16{:}0}$$

PUFA: SFA ratio $= \frac{\sum PUFA}{\sum SFA}$

Omega 6: 3 ratio $= \frac{\sum \omega 6}{\sum \omega 3}$

BIOGRAPHY

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