THE OPTIMUM MIXING RATIO BETWEEN HYDROGENATED PALM KERNEL OIL AND COLD PRESSED RICE BRAN OIL FOR PRODUCTION OF LIQUID AND POWDERED NON-DAIRY CREAMER

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ABSTRACT

Commercially available non-dairy creamer was mainly produced from hydrogenated palm kernel oil (HPKO), which contains trans fat and saturated fatty acid. Consumption of diet high in trans fat and saturated fatty acids can contribute to heart disease and cancer. This research was concerned with substitution of HPKO by cold pressed rice bran oil (CRBO). The objective was to study the optimum mixing ratio between HPKO and CRBO for further utilization in liquid (LNDC) and powdered (PNDC) non-dairy creamer. The physical, chemical and microbiological properties of HPKO and CRBO as ingredients in the production of LNDC and PNDC were studied. The mixing ratios between HPKO and CRBO were statistically designed as 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. The color and viscosity of the mixtures were investigated. As the ratio of CRBO increased, the color became darker (L* of 93.06 to 86.25) while the viscosity of the mixtures of 20:80, 10:90 and 0:100 (54 cp.) were the highest amongst the ratios tested. The HPKO and CRBO mixtures were further chemically tested for fatty acids, γ-oryzanol, α-tocopherol, trans fat contents and antioxidant activity. There were 10 fatty acids present in HPKO with saturated fatty acids being predominant. By contrast, there were only 5 fatty acids found in CRBO with monounsaturated fatty acids being

the major fatty acid. γ-Oryzanol and α-tocopherol contents were higher with increasing CRBO from 0-100% (0 to 1,155.00 mg/100 g oil and 0.09 to 30.82 mg/100 g oil, respectively). Antioxidant activity was increased with increasing CRBO from 0-100% (9.26 to 94.24%). The pure HPKO contained higher trans fat content than that of the 90:10 and 80:20 mixtures (2.73, 1.93 and 1.85 mg/100 g oil, respectively) while no trans fat was detected in other samples. Therefore, substitution of HPKO by CRBO from 30-100% would offer more nutritional value.

The production of LNDC and PNDC was prepared by using CRBO 80 and 90%, respectively. The quality properties and shelf life was studied. The pasteurized LNDC was storage at 4±2°C for 12 days. The physical, chemical, microbiological and sensory properties of the pasteurized LNDC were studied. The color L^*, a^* and b^* were 87.15, -1.59 and 18.39, respectively. The viscosity was 820 cp. γ-Oryzanol and α-tocopherol contents were 792.30 and 4.34 mg/100 g oil, respectively. Antioxidant activity (DPPH) was 50%. There were 8 fatty acids present in the pasteurized LNDC with 6 saturated and 2 unsaturated fatty acids. The pasteurized LNDC had no trans fat and no microorganisms. The shelf life of pasteurized LNDC was 9-11 days. The panelist overall liking score was like moderately-like very much. For PNDC, the condition of shelf life determination was keeping in hot air oven at 30 and 40°C for 80 days. The powder was kept in polypropylene plastic bag. The panelist liking score was about 7-8 (like moderately-like very much).

The cost analysis of products was calculated per one serving price. One unit of LNDC was 250 milliliter in polypropylene plastic bottle (one serving size of 15 mL). The PNDC was keeping 3 gram in polypropylene plastic bag. The price per serving size of LNDC and PNDC was 4.44 and 2.91 baht, respectively.

The marketing and consumer acceptance of both LNDC and PNDC were tested by 400 untrained panelists. The majority of panelists were female. The age was between 20-35 years old. Most of them graduated with Bachelor degree and the salary was higher than 18,000 baht per month. They were college students and lived with family. The coffee taste was the first reason for consumption of non-dairy creamer. 63% of them were interested to buy non-dairy creamer because it is a new product. The appropriate serving size LNDC was 15 ml/ pack in plastic cup with the price of 6-10 Baht, respectively. Whereas for the PNDC was 3 g/50 packs in plastic bag with

the price of 15-30 Baht. The most important factors for buying LNDC and PNDC were price and nutritional value of the products.

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CHARPTER I

INTRODUCTION

Statement of purpose

Rice bran is the cuticle between the paddy husk and the rice grain and is obtained as a by-product of rice processing. Most of the rice bran has been used as a feedstock which has low value. Therefore, there have been attempted to process rice bran into food ingredients and oil sources such as rice bran oil (RBO), protein concentrates and used as ingredients in breakfast cereals which creates potentially value added to rice bran. In addition, there have been many investigations on extraction of importantly high nutritive compounds from rice bran and RBO in order to obtain more valuable products. These compounds include lecithin, vitamin E, γ-oryzanol, inositol and phytin (Naiwikul, 2004).

Rice bran is highly nutritious due to the presence of lipids, protein, minerals and vitamins (B and E). The protein of rice bran is mainly lysine which is found more than bran from other cereals. Furthermore, it contains hypoallergenic protein which is a suitable component for baby food formulation (Wang, et al., 1999). The lipid content of rice bran is 16-32%, with 15-20% saturated fatty acid and 80-85% unsaturated fatty acid. The unsaturated fatty acid, particularly monounsaturated fatty acid can prevent heart disease and some cancers (Wang, et al., 1999).

RBO is a good source of essential fatty acids as linoleic acid (C18:2) 32-38% and linolenic acid (C18:3) 1-2% which consist of high vitamin A, D, E and K, especially total vitamin E (>1,000 mg/kg). It contains monounsaturated fatty acid (MUFA) more than 40% of total fatty acids. This MUFA can reduce low density lipoprotein cholesterol (LDL-C) and increase high density lipoprotein cholesterol (HDL-C) (Amarasinghe and Gangodavilage, 2004). According to its adequate ratio of fatty acids, World Health Organization, American Heart Society, and Food and Agriculture Administration suggested that RBO is appropriate for consumption.

Apart from fatty acids, rice bran composes of vitamins and many important compounds for human health such as vitamin E, γ-oryzanol and tocotrienol which are antioxidants and assist in reducing cholesterol in the body. Moreover, it helps in increasing or maintaining HDL cholesterol which carries cholesterol in cells and blood for combustion.

Tocotrienol presents in large amount in RBO. Apart from having antioxidant activity, it helps in preventing cholesterol synthesis in the body and reduces cardiovascular diseases. In general, RBO contains tocopherol 19-40% and tocotrienol 51-81%. γ-Oryzanol is naturally found in RBO, it does not present in other vegetable oil. It helps in reducing cholesterol in the blood (Nammanboripokethai, 2006).

Non-dairy creamer is the products that does not make from milk and has other fats than cream as ingredients or creamer that contains cream less than 30% (Ministry of Health, 2000). Most of non-dairy creamer makes from coconut and palm kernel oil (PKO), available as powdered, liquid and frozen (Herbst and Wang, 1995) and has the major role to reduce color of coffee and tea and provides flavor. Non-dairy creamer from coconut oil and PKO has high saturated fatty acid (90 and 50%, respectively) while RBO has lower saturated fatty acid (15-20%). This saturated fatty acid, if consume in large quantity may contribute to heart disease and cancer. Moreover, PKO has to import from overseas more than millions Baht a year. For example, in 2004 and 2005, it was imported 1,379 and 2,607 tons, cost 26 and 47 millions Baht, respectively (Department of Custom, 2006). On the other hand, rice bran can be produced domestically about 2 million tons a year in which 70% or 1.4 million tons of rice bran was used for animal feeds and other 15% or 300 thousand tons was processed to RBO which given 45,000 tons of oil a year. However, if all the rice bran has been taken for oil extraction it would yield 300,000 tons a year (Hutapat, 2006) which more than PKO that imported into Thailand. RBO in the market costs 30-40 Baht/kg (Nammanboripokethai, 2006) whereas crude PKO costs 17-25 Baht/kg and has to pass through refining process before consumption which results in 25-30 Baht/kg of PKO available in the market (Department of Agriculture, 2006). This price is similar to that of RBO. After pressing for the oil, the rice bran can still be used for animal feeds with the same price as raw rice bran. Therefore, it can be seen that RBO

could be well replaced or substituted PKO and it is sufficiently available all year round for the industries and also has higher nutritive value than PKO.

This research is, therefore, concentrated on development of LNDC and PNDC from CRBO substituted HPKO to be a new product from rice bran. This creates value added to rice bran and provides an alternative way for coffee and tea drinkers who concern their health in which they can select LNDC or PNDC from RBO substitution which has lower saturated fatty acid and higher unsaturated fatty acid than that of the same products available in the market.

Objectives of the study

The main objective of this research is to find out the suitable mixing ratio between HPKO and CRBO, formula and process for production of LNDC and PNDC from HPKO and CRBO.

The sub-objectives of this research are:

1. To study the properties of HPKO and CRBO and their mixtures.
2. To develop formula and production process for LNDC and PNDC from mixed HPKO and CRBO.
3. To examine the shelf life of the LNDC and PNDC from mixed HPKO and CRBO.
4. To analyze production cost, marketing and consumer acceptance of LNDC and PNDC from mixed HPKO and CRBO.

Expected outputs

1. Find out the suitable mixing ratio between HPKO and CRBO for the production of LNDC and PNDC.
2. Obtain the suitable formula and production process of LNDC and PNDC from HPKO and CRBO.
3. Know the shelf life of LNDC and PNDC from mixed HPKO and CRBO.
4. Know the production cost and consumer acceptance of LNDC and PNDC from mixed HPKO and CRBO.

CHAPTER II

LITERATURE REVIEWS

Non-dairy creamer

Non-dairy creamers are liquid or granular substances intended to substitute for milk or cream as an additive to coffee or other beverages. They do not contain lactose therefore not dairy products; accordingly, some parts of the world require the alternate term non-dairy whiteners that do not imply the presence of real cream (http://en.wikipedia.org/wiki/Non-dairy_creamer). They made with glucose, fat and emulsifying salts. They may be made with casein, in which case it is not technically (or by US law) non-dairy (http://www.encyclopedia.com/doc/1O39-creamernondairy.html).

The main ingredients and their roles in non-dairy creamer (Justo, 2007) are listed below.

1. Corn syrup solids: Corn syrup is, for the most part, glucose. When it is dehydrated to about 10% water, the resulting granules are basically sugar. Both powder and liquid coffee creamer contain corn syrup solids. Corn syrup solids are the crystallized form of corn syrup. It is created by adding water and a small amount of hydrochloric acid to corn starch. The mixture then undergoes a heating process under pressure where the starch molecules break down and convert to sugar. The solid form is an evaporated form of this. This is not a form of high fructose corn syrup, which requires additional processing to increase sweetness.

2. Vegetable oil solids: Powdered creamer has to get its creamy texture somewhere. Coconut oil and PKO are among the heavier food oils and are added, in all their partially hydrogenated glory, for velvety smoothness. Because they add up to less than 0.5 gram of trans fat, the label can claim "zero grams trans fat."

3. Sodium caseinate: Casein is a protein found in cow's milk, thus making this non-dairy product off-limits to vegans. Officially, it is Kosher, but do not go mixing it with meat. Sodium caseinate is a milk-derived protein present in many non-dairy products. The additive originally comes from milk, but the chemical conversion process leaves its molecular makeup different from its original form. The main

purpose of including sodium caseinate in creamer is to provide a dairy flavor and also to give the creamer a thick and creamy appearance. There is some controversy in the Jewish community as to whether Coffee-Mate and other non-dairy food products that contain sodium caseinate are Kosher. Some Orthodox practitioners argue that because it is originally derived from milk, it is still a dairy product, even though both dairy scientists and government regulators say it is not.

4. Dipotassium phosphate: Also called phosphoric acid, dipotassium phosphate provides the tang in Coca-Cola. It helps to digest sugars, fats and proteins, which happen to be non-dairy creamer's top three ingredients.

5. Monoglycerides and diglycerides: These single- or double-chain fatty acids end in a glycerol molecule. The glycerol end attaches to water and the fatty acid end to fats and oils, making these substances gentle mediators between the creamer and the coffee.

6. Sodium alumionosilicate: Known to potters as albite feldspar (a ceramic glaze and strengthening agent), sodium alumionosilicate is used in foods as an anti-caking agent. PNDC is flammable and if it gets too anticaked (dispersed in the air like a cloud), it can explode. Sodium aluminosilicate is a compound which may include sodium, aluminium, silicon and oxygen. The compound also contains synthetic zeolites, which are anti-caking agents. Both the powdered and liquid product contains sodium aluminosilicate. The compound does not have any significant health risks associated with it.

7. Artificial flavors: Since glucose, PKO and sodium caseinate do not really taste like cream, artificial flavors are required. Manufacturers tend to keep exactly what they use a secret. These flavors can sometimes come in very non-cream varieties like hazelnut, amaretto, or mocha.

8. Annato: This natural pigment from a tropical plant provides a yellow color, so the creamer looks more, dairy-ish.

Non-dairy creamer comes in powder form or as a refrigerated liquid. Advantages of the powder form are that it will not cool the coffee and it has a longer shelf life. However, some find LNDC more convenient and richer in texture and taste. Generally, it is recommended that LNDC be consumed within 14 days of opening the product (http://www.wisegeek.com/what-is-non-dairy-creamer.htm).

CRBO was initially used for production of powdered non-dairy creamer by Singanusong and Noitup (2009). The cold pressed oil was filtered, passed through UV lamp and magnetic field and finally degummed before using as one ingredient for production of powdered non-dairy creamer using the spray dryer. The powdered non-dairy creamer from RBO had similar quality to that made from PKO. Therefore, RBO could be used to make powdered non-dairy creamer instead of PKO which had to import from overseas. However, the shelf life of the product needed to be further investigated.

Spray drying

Spray drying is a one-step processing operation for turning a liquid feed into a dried particulate form, by spraying the feed into a hot drying gas medium. While reduces the product bulk weight and size, spray drying minimizes handling and also preserves the product by reducing its water activity to a low level, required to stop bacterial degradation (Hayashi, 1989; Chiou and Langrish, 2006). The production of dried particles from a liquid feed in a single processing step makes spray drying an exceptional and important process. Spray drying is widely applied in several industrial sectors including the food, pharmaceutical and chemical. Several biological and thermal-sensitive materials, liquid materials, such as milk, fruit juices and pulps, herbal extracts, enzymes, essential oils, aromas and various pharmaceuticals have been dried by this process (Masters, 1972; Barbosa-Canovas, et al., 2005). Its adequacy to process thermal-sensitive materials is mainly due to the short residence time of the product inside the dryer (in the order of a few seconds). One of the most remarkable advantages of the spray drying is the capacity of process, several kinds of materials and the possibility of obtain a dried product with pre-specified properties. These characteristics are particularly important when the dried products of good quality and high content of healthy enhancing substances, such as the phenolic compounds, flavonoids, carotenoids and so on to be obtained.

Drying is generally applied in food and nutraceutical processing, aiming the reduction of the product water activity to a safe level, which assures its microbial stability and minimizes physical and chemical changes during storage. Several drying systems can be employed for dehydration of foods and nutraceuticals, including

vacuum drying, convective drying, freeze drying, spout and fluidized bed drying and spray drying. The selection of a particular equipment depends on the type and properties of the material to be processed as well as the desired product properties.

Spray drying is highly suitable for the continuous production of dry powders, granules or other agglomerated products from liquid pump able feedings, such as solutions, suspensions and emulsions. The concept of spray drying is based on the high increase in the surface area of the contact area between the material to be dried and the drying medium promoted by the atomization. Different spray drying configurations exist, which varies in size and shape of the drying chamber, type of atomizing devices (rotary atomizers, pressure nozzles, pneumatic nozzles, ultrasonic devices and so on), air-droplet contact system (co-current, counter-current and mixed flow) and product collecting systems.

Interesting reviews of the spray drying technique, focusing in hardware and process parameters as well as in current applications on pharmaceutical technology were presented by Krzystof and Krzystof (2009a; Krzystof and Krzystof, 2009b). The design of a spray drying process includes the establishment of operating conditions that increase product recovery and produce an end product of a predefined quality specification (Langrish, 2007). The short residence time of the product inside the dryer makes the spray drying suitable for the processing of themosensitive materials, including pharmaceuticals and biological materials. The spray drying is widely used in the preparation of dried powders from extracts of medicinal plants, fruit pulps, plants oleoresins, essential oils and so on (Wiesenborn, Zbikowski and Nguyen, 1995; Edris and Bergnstahl, 2001; Soares, et al., 2005; Fernandes, et al., 2008; Souza, et al., 2009; Acosta-Esquijarosa, et al., 2009; Angel, et al., 2009; Wang, et al., 2009).

Rice bran

Rice bran is the cuticle between the paddy husk and the rice grain (the starchy endosperm) and is obtained as a by-product of rice processing (Grist, 1985; Saunders, 1985). It has been used as a feedstock and has the potential to be used as a food ingredient and oil source (McCaskill and Zhang, 1999). The bran is highly nutritious due to the presence of lipids, protein, minerals and vitamins. The composition of rice bran varies with the rice type, climatic conditions and rice processing methods (Grist,

1985). The oil content in rice bran varies from 12 to 25% (Pillaiyar, 1980; Saunders, 1985) and approximately 95–98% of the oil is extractable (Pillaiyar, 1980). Rice bran has low moisture content (6–7%) and possesses a powdery consistency (Saunders, 1985). Rice bran also contains a high level of dietary fibers and B-complex vitamins (Goenka, 1987; Moreau and Powell, 1999; Anderson and Guraya, 2001; Gopala Krishna, Khatoon and Babylatha, 2005; Anil Kumar, et al., 2006; Kadam and Bhowmick, 2006; Yu, et al., 2006).

Composition of rice and rice bran lipids

1. Lipid

The structure of the rice kernel is given in Figure 1. Lipids are present as spherosomes or lipid droplets less than 1.5 mm in diameter in the aleurone layer, less than 1.0 mm in the subaleurone layer and less than 0.7 mm in the embryo of the rice grain (Juliano, 1983a; Godber and Juliano, 2003). Most of the lipids in the endosperm are associated with protein bodies and the starch granules as bound lipids (Morrison, 1978). The lipids are broadly classified as nonstarch and starch lipids (Table 1). The majority of the lipids are the nonstarch lipids. Starch lipids consist primarily of lysophospholipids, triacylglycerols and free fatty acids (Morrison, 1988). Major phospholipid species are lysophophatidylethanolamine and lysophosphatidylcholine. The major fatty acids are palmitic and linoleic acids along with oleic acid. Minor amounts of monoacylglycerols, diacylglycerols and sterols are also found. Glycolipids found are diglycosyl monoacylglycerols and monoglycosyl monoacylglycerols. The component sugars are galactose and glucose. The nonstarch lipids in the aleurone, subaleurone and germ layers were 86–91% neutral lipids, 2–5% glycolipids and 7–9% phospholipids, although these are variable because of different milling degrees (Choudhury and Juliano, 1980). The fatty acid composition of nonstarch lipids showed 22–25% palmitic, 37–41% oleic acid and 37–41% linoleic acid (Table 2).

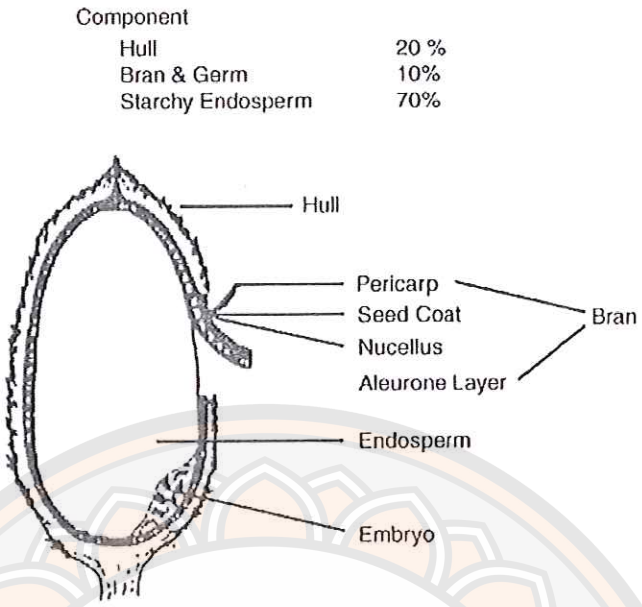

Figure 1 Relative proportion of major rice caryopsis components

Source: Orthoefer, 2001

Table 1 Lipid composition of rice and its fractions[a]

Property	Nonstarch Lipids in Rice Fractions						Nonwaxy Starch Lipid in Rice Fractions	
	Hull	Brown	Milled	Bran	Germ	Polish	Brown Rice	Milled Rice
Lipid content	0.4	2.7	0.8	18.3	30.2	10.8	0.6	.05
Saponification no.	145	181	190	184	189			
Iodine no.	69	94	100	99	101			
Unsaponifiable matter	26	6	6	6	34			
Fatty acid composition Wt % of total								

Table 1 (cont.)

Property	Nonstarch Lipids in Rice Fractions						Nonwaxy Starch Lipid in Rice Fractions	
	Hull	Brown	Milled	Bran	Germ	Polish	Brown Rice	Milled Rice
Palmitic	18	23	33	23	24	23	46	45
Oleic	42	35	21	37	36	35	12	11
Linoleic	28	38	40	36	37	38	38	40
Others	12	4	6	4	3	4	4	4
Neutral lipids, % of total lipids	64	86	82	89	91	87	28	26
Triglyceride	-	71	58	76	79	72	4	2
Free fatty acids	-	7	15	4	4	5	20	21
Glycolipids, % of total lipids	25	5	8	4	2	5	19	16
Phospholipids, % total lipids	11	9	10	7	7	8	53	58
Phosphotidylcholine	-	4	9	3	3	3	4	4
Phosphatidylethanolamine	-	4	4	3	3	3	5	5
Lysophosphatidylcholine	-	<1	2	<1	<1	<1	21	23
Lysophosphatidylethanolamine	-	-	1	-	-	-	22	25

Note: [a] Based on 6% bran-germ, 4% polish and 90% milled rice from brown rice.

Source: Choudhury and Juliano, 1980; Juliano, 1983a; Juliano, 1983b; Godber and Juliano, 2003

The brown rice nonstarch lipids were 14–18% in germ, 39–41% in bran, 15–21% in polish and 25–33% in milled rice. The composition was 83–87% triacylglycerol together with 7–9% free fatty acids, diacylglycerols, sterols together with sterol esters, hydrocarbons and wax. Oil extracted from rice bran contained 20.1% total lipid, 89.2% neutral lipids, 6.8% glycolipid and 4.0% phospholipid (Shin and Godber, 1996). A component of RBO that has promise as a nutraceutical compound is γ-oryzanol (Rukmini and Raghuam, 1991).

Table 2 Major lipid classes of crude bran oil extracted from raw rice bran and their fatty acid composition

		Fatty Acid Composition (%)								
Lipid class[a]	Wt%	14:0	16:0	18:0	18:1	18:2	18:3	20:0	Saturated	Unsaturated
TL	20.1	0.40	22.21	2.21	38.85	34.58	1.14	0.61	25.43	74.57
NL	89.2	0.43	23.41	1.88	37.24	35.29	1.07	0.68	26.40	73.60
GL	6.8	0.09	27.34	0.28	36.45	35.76	0.18	-	27.61	72.39
PL	4.0	0.11	22.13	0.16	38.11	39.32	0.17	-	22.40	77.60

Note: [a]TL = total lipids; NL = neutral lipids (nonpolar lipid and free fatty acids); GL = glycolipids; PL = phospholipids.

Source: Shin and Godber, 1996

2. γ-Oryzanol

γ-Oryzanol was first isolated from soapstock from rice oil refining (Seetharamaiah and Prabhakar, 1986). Although originally thought to be a single compound, it is now known to be a mixture of steryl and other triterpenyl esters of ferulic acids (cycloartenyl ferulate, 24 methylenecycloartenyl ferulate, β sitosterol ferulate and campesteryl ferulate) (Figure 2). It is present at 1.5–2.9% of RBO with a melting point of 138.5°C. The oryzanol content is dependent on rice grain variety with long grain rice at 6.42 mg/g and medium grain rice at 5.17 mg/g (Lloyd, Siebenmorgen and Beers, 2000).

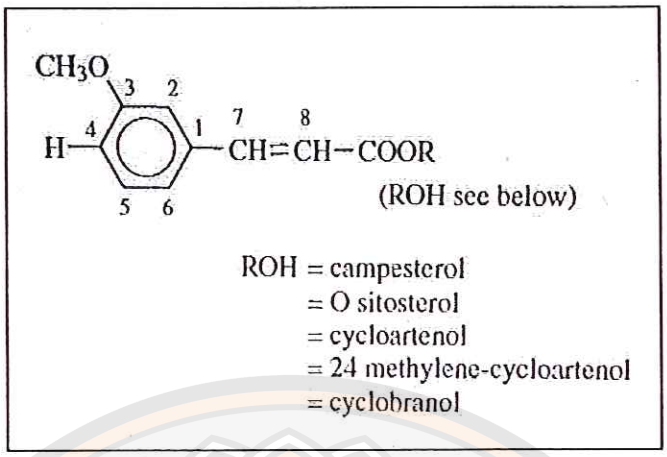

Figure 2 Major ferulates in oryzanol

Source: Godber and Juliano, 2003

3. Tocopherols and tocotrienols

Tocopherols and tocotrienols (tocols) are present in rice oil (Figure 3). Crude RBO was found to contain, per 100 g of oil, 19–46 mg of α-tocopherol, 1–3 mg of β-tocopherol, 1–10 mg of γ-tocopherol and 0.4–0.9 mg of δ-tocopherol, 14–33 mg of α-tocotrienol and 9-69 mg of γ-tocotrienol (Tanabe, Yamaoka and Kato, 1981; Tanabe, Yamaoka and Kato, 1982) (Table 3). The mean tocol content was 93 mg/100 g for crude oil and 50 mg/100 g for refined oil (Tanabe, Yamaoka and Kato, 1982). However, close to 370 mg/100 g has been reported (Gingras, 2002). Rice bran stabilization and storage (Shin, et al., 1997) and method of extraction (Hu, et al., 1996) affects the concentration of tocols in the oil. γ-Tocotrienol is more stable and persists to a greater extent during storage than other tocols (Shin, et al., 1997). Other factors influencing tocols content are milling and variety. Long-grain varieties have higher levels of tocotrienols than medium grain rice (Lloyd, Siebenmorgen and Beers, 2000).

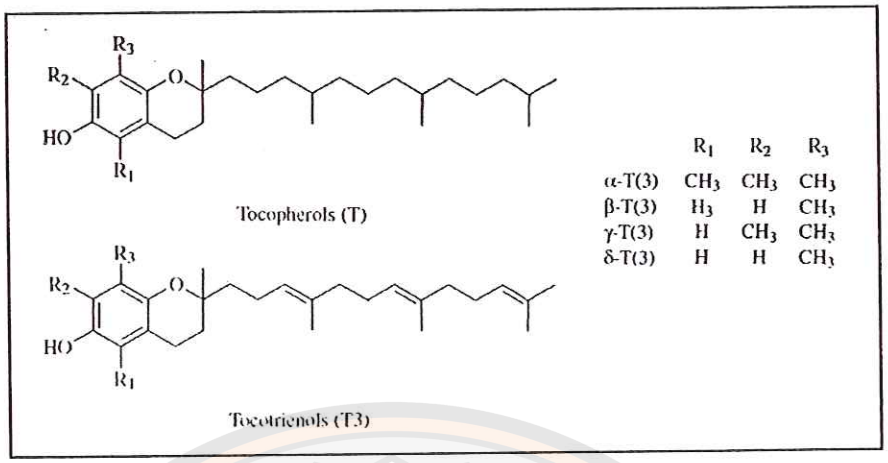

Figure 3 Structure of tocopherol and tocotrienol

Source: Godber and Juliano, 2003

Table 3 Tocopherol and tocotrienol concentrations (mg/100 g) in brown rice bran and commercially available refined oil

Source	α-T	β-T	γ-T	δ-T	α-T3	γ-T3	δ-T3
Rice bran	6.3	0.9	3.20	0.20	3.8	12.0	0.7
Brown rice[a]	0.63	0.09	0.32	0.02	0.38	1.2	0.07
Crude oil[a]	31.50	4.50	16.00	1.00	19.0	60.0	3.5
Refined oil	8.2	-	12.80	1.3	2.1	42.9	3.5

Note: [a]Calculated values.

T= Tocopherol, T3= Tocotrienol

Source: Shin and Godber, 1996

4. Waxes

Waxes are present as long-chain fatty acid esters with fatty alcohols, methanol and ethanol. Fatty acid analysis showed that behenic (C:22), lignoceric (C:24) and palmitic acids (C:16) are the major fatty acids for longer alkyl esters and oleic and palmitic for the shorter alkyl esters (Table 4) (Silto, Susuki and Fujino,

1981). The major alcohols found are for longer alkyl esters. These are tetratriacontanol (C34:0), triacontanol (C30:0), dotriacontanol (C32:0), octacosanol (C28:0) and tetracosanol (C24:0).

Table 4 Typical fatty acids composition of RBO

Fatty Acids	Weight (%)
C14:0	Trace amount
C16:0	16
C18:0	2
C18:1	42
C18:2	38
C18:3	1.4
C20:0	0.6

Source: Hargrove, 1994

5. Other compounds

Straight-chain alkanes, alkenes and branched-chain alkenes (squalene) are detected in the hydrocarbon fraction. The squalene content is 120 mg/100 g. Hard and soft waxes are recovered from crude RBO with melting point of 79.5°C and 74°C (Yoon and Rhee, 1982). The hard wax consists of 64.5% fatty alcohols, 33.5% fatty acids and 2% hydrocarbons. Soft wax includes 51.8% fatty alcohols, 46.2% fatty acids and 2% hydrocarbons.

Rice bran oil (RBO)

RBO is the oil extracted from the germ and inner husk of rice. It is notable for its very high smoke point of 490 F (254°C) and its mild flavor, making it suitable for high-temperature cooking methods such as stir frying and deep frying. It is popular as a cooking oil in several Asian countries, including Japan and China. It contains a range of fats, with 47% of its fats monounsaturated, 33% polyunsaturated and 20% saturated. It presents unique health benefits that may be attributed to its high level of

unsaponifiable matter, whose most important components are γ-oryzanol, a complex mixture of ferulate esters with sterols and triterpene alcohols (Kim, et al., 2001) and tocopherols/tocotrienols, a family of isomers that presents vitamin E activity (Shin, et al., 1997; Kim, et al., 2001).

RBO is widely used in pharmaceutical, food and chemical industries due to its unique properties and high medicinal value (Amarasinghe, Kumarasiri and Gangodavilage, 2009). RBO contains natural antioxidants such as tocopherol and oryzanol which contribute to the lowering blood serum cholesterol, have anti-cancer properties and protect the body from free-radical damage (Anderson and Guraya, 2001; Gopala Krishna, Khatoon and Babylatha, 2005; Anil Kumar, et al., 2006; Kadam and Bhowmick, 2006; Yu, et al., 2006).

RBO has high medicinal value and hence is used in many pharma-ceutical manufacturing processes (Adhikari and Adhikari, 1986). Non-edible RBO is used in products such as cosmetics, paints, soaps and detergents (Thirumala Rao, 1961; Soares, 1987). Edible RBO is also a good substitute for vegetable oils (Sayre, Nayyar and Saunders, 1985; Goenka, 1987).

The high oil content of bran makes it subject to rancidification, one of the reasons that the bran is often separated from the grain before storage or further processing. The bran itself can be heat-treated to increase its longevity. Chemical stabilization or refrigeration can also be used for controlling the lipase activity (Prabhakar and Venkatesh, 1986; Anil Kumar, et al., 2006). Extraction and pressing are the commonly used methods for the separation of oils from raw materials (Proctor, et al., 1994; Proctor and Bowen, 1996). Super critical extraction has been experimented for extraction of oils from natural materials (Kuk and Doud, 1998; Perretti, et al., 2003; Abdulkarin, et al., 2006; Lau, et al., 2006). Many researchers have investigated on the use of enzymes for enhancing oil extractability (Bargale, Sosulski and Sosulski, 2000; Hanmoungjai, Pyle and Niranjan, 2001; Sharma, Khare and Gupta, 2001; Puangsri, Addulkarim and Ghazali, 2005).

Orthoefer (1996) reported that the bran and polish, the source of RBO was derived from the outer layers of the rice caryopsis during milling. RBO usually contains palmitic, oleic and linoleic fatty acids constituting more than 90% of the fatty acid portion of glycerides (Table 4). However, the major molecular species of RBO

triglycerides are palmitic-linoleic-oleic, oleic-linoleic-palmitic, palmitic-linoleic-linoleic, linoleic-linoleic-palmitic and finally triolein.

Rice bran contrains several enzymes. Lipase has been the major type of enzyme and affects the keeping quality and the subsequent industrial usage of the rice bran lipase predominately promotes hydrolysis of the oil in bran into glycerol and free fatty acid (FFA). The rate of FFA development is quite high and directly depended on environmental condition. FFA developed about 5-7% per day and up to 70% FFA for a single month during storage (Orthoefer, 1996).

Hargrove (1994) reported that there were many potentially suitable methods to stabilize or inactivate the lipase in rice bran. Most commercial systems currently utilized in the United State employed the moisture added or dry extrusion methods. The bran temperature maintained at 90-100°C, after extrusion for 2-3 minutes prior to cooling. After the extrusion stabilization, produced bran would remain stable under normal warehouse storage for maximum six months but refrigerated storage was able to extend the shelf life significantly.

Nicolosi, et al. (1994; Orthoefer, 1996) found that oil easily removed from the bran using hydraulic pressing and/or solvent extraction (Figure 4). Extraction of the oil may be carried out with a variety of solvents, although hexane is generally used. Rice bran for solvent extraction may be steamed for stabilization and to facilitate pellet or collate formation for higher solvent per collation rate leading to shorter extraction time. The extraction usually may be divides into a batch type or continouous extractor. The solvent plus oil, referred to as micelle, is filtered prior to distillation of solvent. The wet defatted bran is desolvented, dried and cooled. The solvent is recovered throughout the process. However, crude RBO is dark greenish brown to light yellow, depending on the condition of the bran, extraction method and composition of the bran.

The products of extraction may consist of defatted bran, crude RBO, wax and soap of fatty acid. In refining process (Figure 5), the processes used to preparation of food oils consist of dewaxing, the simplest technique to remove the wax from crude RBO is to use settling tanks in which the crude oil is gradually cooled, followed by filtering or centrifuging (Orthoefer, 1996).

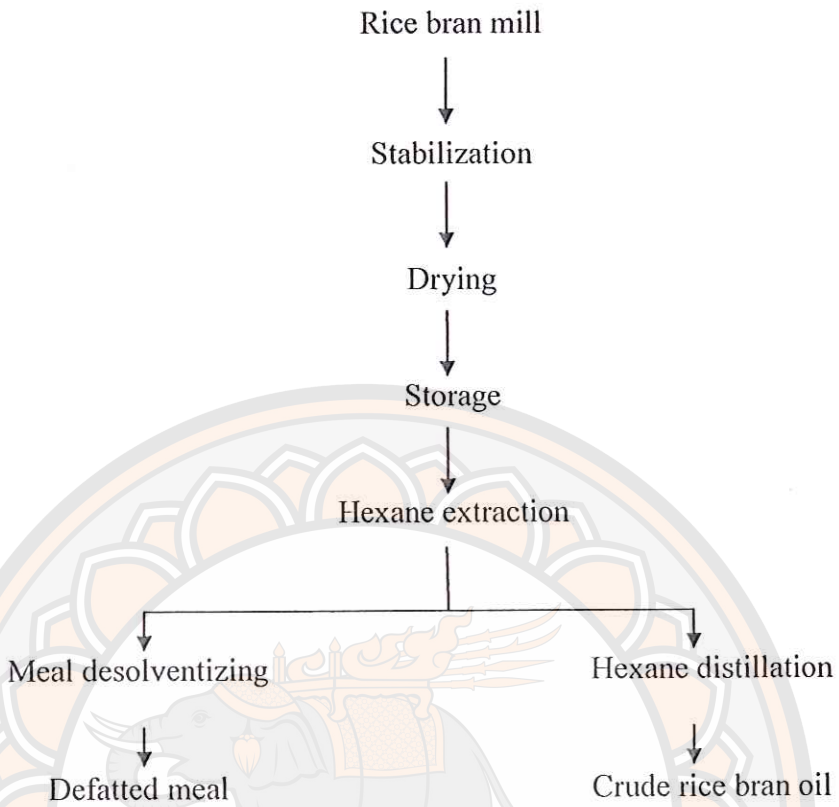

Figure 4 Rice bran oil extraction process

Source: Nicolosi, et al., 1994

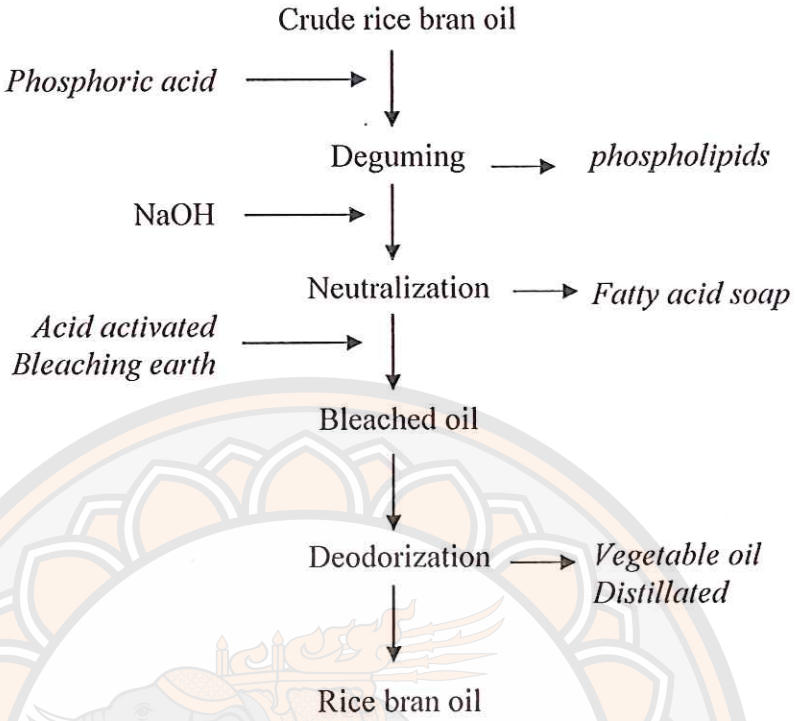

Figure 5 Rice bran oil refining process

Source: Nicolosi, et al., 1994

Refined rice bran oil (RRBO) or commercial RBO is extracted using organic solvents (Johnson and Lusas, 1983). Hexane has been used as the solvent for rice bran extraction by many researchers and industrialists due to the availability, high oil extractability (98%) and easy operation (Johnson and Lusas, 1983). This process, however, has some problems with respect to the oil quality. The hexane extracted crude oil has a high content of free fatty acid, wax and unsaponifiable matter and also suffers from a dark greenish-brown to light yellow coloration (Minsh, Kakar and Surnua, 1990; Krishna, 1992; Yoon and Kim, 1994). The extracted oil therefore requires further refining. Furthermore, hexane, which can be emitted during extraction and recovery, has been identified as an air pollutant because it can react with other pollutants in the atmosphere to produce ozone and photochemical oxidants (Hanmoungjai, Pyle and Niranjan, 2001).

Nicolosi, et al. (1994) presented that the degumming process generally used of degumming agents such as phosphoric or citric acid to hydrolyze at 60-80°C. The wet gum was separated from the oil by centrifugation. Acid degumming was usually combined with neutralization with sodium hydroxide, at temperature less than 65°C. Free fatty acids presently were converted to sodium soaps, being hydratable and removable by centrifugation. Bleaching of the oils was carried out to remove pigments, oxidized lipids and polar component from the oil. Acid activated bleaching clay was removed by filtration. Finally, to remove of odors, flavors and fatty acid by steam distillation or deodorization at 220–225°C, 4–8 mmHg. The volatile compounds including aldehydes, ketones and peroxide could be removed from oils. After deodorization, the RBO was cooled to 10–14°C prior to storage (Table 5).

Table 5 Characteristic of refined RBO

Characteristic	Quality
Iodine value	99-108
Saponification value	180-190
Smoke point	213°C
Fire point	352°C
Cloud index	17°C
Refractive index 25°C	1.470-1.473
Specific gravity 25/25°C	0.916-0.921
Unsaponifiable matter	3-5%
Total tocopherol	200 mg/kg (0.02%)

Source: Hargrove, 1994

Winterization was performed to remove the high melting triglyceride from the fractions, the oil remained liquid at refrigeration temperature. The oils were winterized by slowly cooling the oil to 5°C and holding for up to several days. The saturated glyceride crystallize could be removed by filtration producing a stearin (high melting fraction) and rice oil (low melting fraction) (Nicolosi, et al., 1994). The

composition of RBO suggested that it could be used as a salad oil and for cooking. Therefore, it had excellent oxidation stability, because it contained the natural tocopherol (Sonntag, 1979).

Roger, et al. (1993) and Diack and Saska (1994) found that fully processed RBO contained a high amount of unsapoinfiable component compared to most other vegetable oils. Two groups of components found in the unsaponifiable fraction of rice bran have been investigated for possible health benefit, such as the tocotrienol and the γ-oryzanol. Although, their concentrations substantially depended on the origin of the rice bran.

RBO was used for both edible and industrial application. Only high quality RBO was used for foods. The oxidative stability of RBO was equivalent to or better than soybean, corn, canola, cottonseed and safflower oils in deep frying condition. Winterized RBO was suitable for making mayonnaise and salad dressing. The stearin separated during winterization could be used in margarine and shortening application (Orthoefer, 1996).

Cold pressed rice bran oil (CRBO) is exceptionally nutritious and rich source of valuable minor nutraceutical components such as γ-oryzanol, tocopherol, tocotrienols, phytosterols, polyphenals and squalene. These bioactive unsaponifiable compounds can be found particularly in CRBO, which has received more attention and become more profitable in Thailand, Malaysia and Japan as food supplement, main ingredient for nutrition, cosmetics and pharmacy and for extraction of those bioactive components. CRBO is produced without chemicals and successive steps of refining, except membrane filtration whereas RRBO oil involve using chemicals, heat and cold condition in order to improve quality of the oil. Without severe condition, CRBO retains much more concentration of such minor compounds than those RRBO (Singanusong, 2012).

Palm oil

Palm oil is derived from the mesocarp of the palm fruit, species *Elaeis guineesis*. Presently, palm oil is now the second largest vegetable oil in the world production and the leader in the world exports (Pantzaris, 1995). In generally, palm oil had reddish brown in color due to it was high content of carotenoid, α and β carotene

about 500-700 ppm. During refining process, the β-carotene was gradually decreased. Palm oil has a semi-solid consistency at ambient temperature, due to containing about 50% unsaturated fatty acids. The chain lengths of fatty acids presented in the triglyceride comprising of a very narrow range from 12 to 20 carbon atoms (Basiron, 1996).

Sambanthamurthi, Sundram and Tan (2000) reported that over 95% of palm oil consisted of the mix triglycerides containing glycerol molecules as the backbone and each of molecule esterified with the three fatty acid. The major fatty acids in palm oil were myristic, palmitic, stearic, oleic and linoleic (Table 6). Goh (1991) reported that two major components of triglycerides in palm oil were unsaturated-dipalmitin (C50) and palmitounsaturated (C52) (Table 7). Normally C50 included palmitic-oleic-palmitic (POP) and palmitic–palmitic–oleic (PPO) and C52 was palmitic–oleic–oleic (POO) (Basiron, 1996). However, the triglycerides in palm oil partially exhibited the physical characteristics of the palm oil such as melting point and crystallization behavior (Sambanthamurthi, Sundram and Tan 2000).

Sambanthamurthi, Sundram and Tan (2000) also reported that the minor constituents of palm oil could be divided into two groups. The first group consisted of fatty acid derivatives, such as partial glycerides (mono-and diglycerides), phosphatides, ester and sterols. The second group included classes of compounds not related chemically to fatty acid, such as hydrocarbons, aliphatic alcohols, free sterols, tocopherol, pigments and trace metals. Most of the minor components found in the unsaponnifiables fraction of palm oil were sterols, higher aliphatic alcohol, pigments and hydrocarbons. In addition, palm oil contained mainly three types of diglyceride, C32 (dipalmitoylglycerol or PP), C34 (palmitoyloleoyglycerol or PO) and C36 (dioleoylglycerol or OO). The diglycerides in palm oil affected its physical property such as crystallization (Table 9).

Basiron (1996) reported that the extraction processes of palm oil usually began with the fruit reception, sterilization, stripping, digestion, oil extraction, clarification and oil storage, respectively (Figure 6). Crude palm oil was extracted commercially impurities, such as mesocarp fibers, moisture and insoluble, free fatty acid, phosphatides, trace metals and oxidation products. As the result, palm oil was

normally refined to bland, stable product before used for consumption or formulation of edible product (Figure 7).

Table 6 Fatty acid composition of palm oil

Fatty acid	Weight (%)		
	Symbol	Mean	Range
Saturated Acids			
Lauric	C12:0	0.2	0.1-0.3
Myristic	C14:0	1.1	1.0-1.3
Palmitic	C16:0	44.7	43.9-46.0
Stearic	C18:0	4.2	3.9-4.4
Arachidic	C20:0	0.4	0.3-0.7
Mono-unsaturated Acids			
Palmitoleic	C16:0	0.1	0-0.1
Oleic	C18:0	39.2	38.0-40.6
Poly-unsaturated Acids			
Linoleic	C18:2	10.0	9.2-10.5
Linolenic	C18:3	0.3	0.3-0.6

Source: Goh, 1991

Table 7 Triglyceride composition of crude palm oil

Triglycerides	Weight (%)	
	Mean	Range
C46	0.8	0.4-1.2
C48	7.4	4.7-10.8
C50	42.6	40.0-45.2 (POP, PPO)
C52	40.5	38.2-43.8 (POO)
C54	8.0	6.4-11.4

Source: Goh, 1991

Basiron (1996) reported that two methods of refining, namely physical and chemical refinings, were available to refined crude palm oil (Table 8). However, physical refining has become the major processing route because of cost effective, high efficiency and simple effluent treatment. However, both processes were able to produce refined, bleached and deodorized (RBD) palm oil of desirable quality and stability for edible purposes.

Table 8 Refining crude palm oil: unit process

Stage	Principal impurities reduced or removed
Degumming	Phospholipids, trace metals, pigments
Neutralization	Fatty acid, phospholipids, pigments, oil insoluble, water soluble
Washing	Soap
Drying	Water
Bleaching	Pigments, oxidation products, trace metal, traces of soap
Filtration	Spent bleaching earth
Deodorization	Fatty acids, mono-and diglyceride, oxidation products, pigment
Physical refining	Fatty acids, mono-and diglyceride, oxidation products, pigment
Polishing	Removal of trace oil insoluble

Source: NorAini, Abdullahs and Halim, 1992

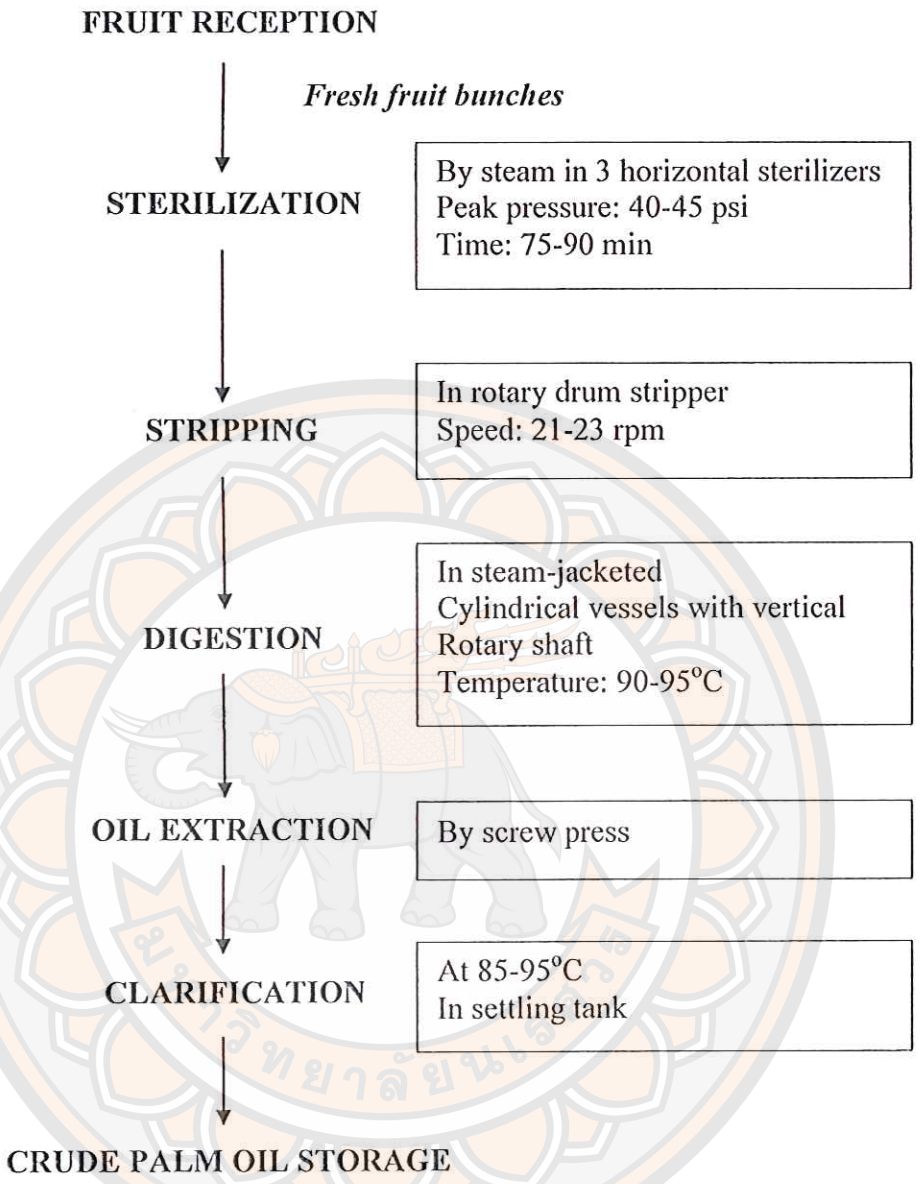

Figure 6 Extraction of crude palm oil

Source: NorAini, Abdullahs and Halim, 1992

Table 9 Major physical properties of palm oil

Property	Mean	Range
Apparent density at 50°C g/ml	0.889	0.888-0.889
Refractive index at 50°C	1.455	1.455-1.456
Solid fat content (%)		
At 5°C	60.5	50.7-68.0
At 10°C	49.6	40.0-55.2
At 15°C	34.7	27.2-39.7
At 20°C	22.5	14.7-27.9
At 25°C	13.5	6.5-18.5
At 30°C	9.2	4.5-14.1
At 35°C	6.6	1.8-11.7
At 40°C	4.0	0.0-7.5
At 45°C	0.7	-
Slip point (°C)	34.2	31.1-37.6

Source: Sambanthamurthi, Sundram and Tan, 2000

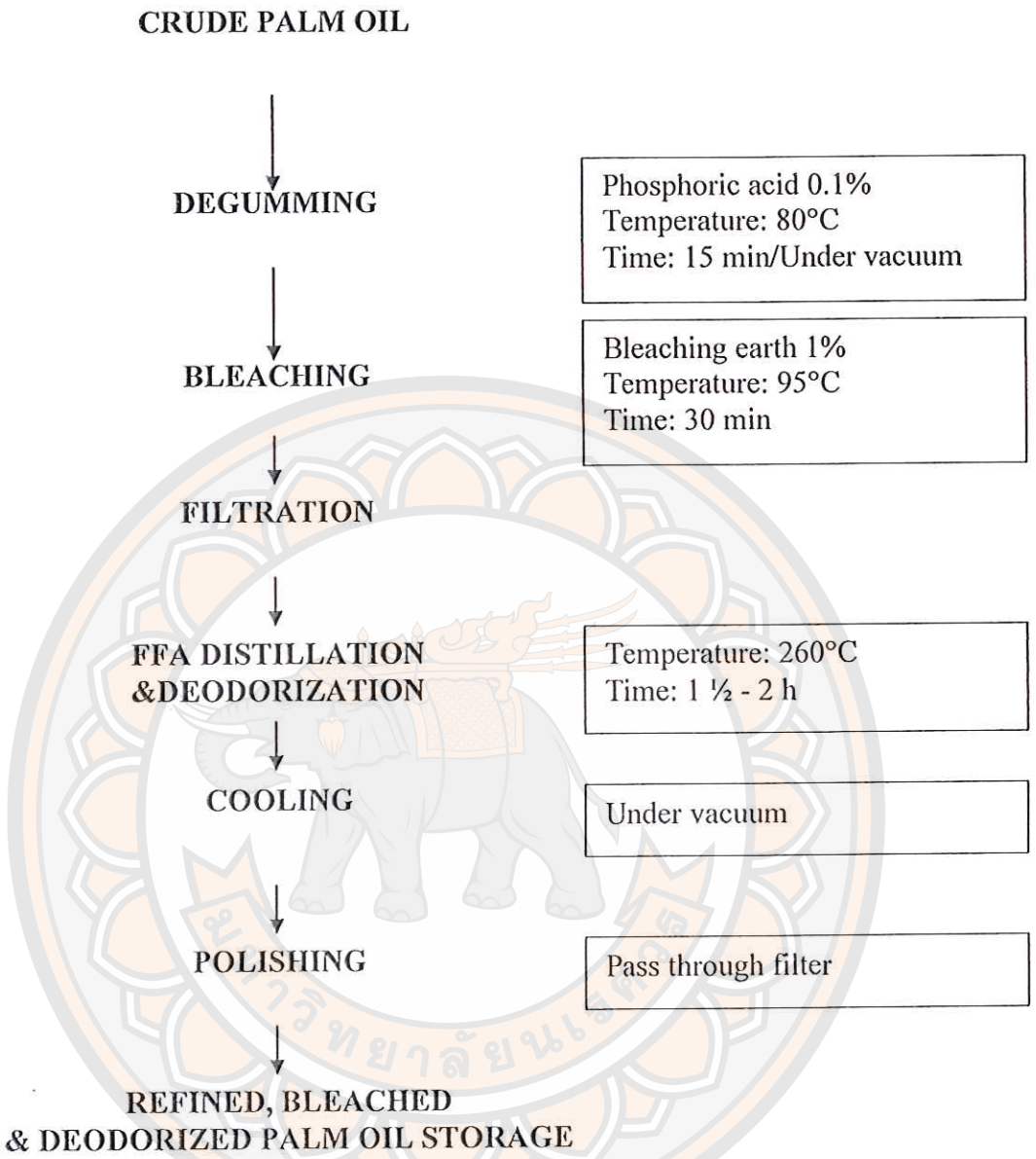

Figure 7 Refining of palm oil

Source: NorAini, Abdullahs and Halim, 1992

Blended oil

The blending, mixing two or more straight or modified oils and fats, could be the correct balance of properties such as melting point, plastic range, color, texture, iodine value, etc. (Berger, 1982). Consequently, blending was the simplest method used to modify oils and fats for some specific applications (NorAini, et al., 2001).

In tropical countries, palm olein were sold in supermarket or retail shops for general household cooking and frying which might have a preference for a particular flavor and taste. Palm olein could easily be blended with other oils such as groundnut oil and sesame oil that had proved to be very popular in China as household frying oils. The blends of palm olein with soybean oil provided oil with a balance ratio of polyunsaturated, monounsaturated and saturated fatty acids as recommended by some health organizations. The new cooking oil was recently introduced in the local market under the brand name of DAISY. This cooking was the blend of palm olein with sunflower seed oil and canola oil (Razali and NorAini, 1994).

In Egypt, cottonseed oil was the major vegetable oil. It comprises 79.6% of the total oil production. Recently, palm olein has been intensively used in Egypt as a frying medias due to high oxidative stability and low price as compared to other similar frying media. Blending of cottonseed oil with palm olein also improved the frying performance (Mostafa, et al., 1996). However, the palm olein was blended with high polyunsaturated fatty acid such as soybean oil and sunflower oil to increase the cold stability and frying performance (Chu, 1991; Basonglu, et al., 1996)

In 1992, Uniliver in Italy using palm olein as the main component blended with sunflower seed oil and groundnut oil. The product was sold in Italian supermarkets under the brand name of FRIOL. Blending of palm olien with other polyunsaturated oil was a good practice because the final blended oil had a better frying performance when compared with the polyunsaturated oil alone (Razali and NorAini, 1994). However, there has been no information on blended HPKO and RBO.

CHAPTER III

RESEARCH METHODOLOGY

This chapter presents the methodology of this research including materials, chemical, apparatus and methods. The details of each topic are described below. Furthermore, in order to achieve the objectives of this research, the research methodology was divided into 4 parts.

Materials

1. Hydrogenated palm kernel oil (HPKO), by Korn Thai Co., Ltd., Ratchaburi province, Thailand
2. Cold pressed rice barn oil (CRBO), by Kiatsiri Pharmacy, Lopburi province, Thailand
3. Coffee (Nescafe brand)
4. Sugar (Aro brand)
5. Powder non-dairy creamer (Nescafe brand)
6. Liquid non-dairy creamer (Nescafe brand)
7. Lecithin, LECICO GmbH, Germany
8. Sodium caseinate, Lactoprot, Germany
9. Moltodrextrin (DE 10), CP Kelco ApS, Denmark
10. Glucose syrup, Union Science, Thailand

Chemicals

1. Acetic acid-chloroform solution (Sigma-Aldrich, USA)
2. Potassium iodide solution (Sigma-Aldrich, USA)
3. Sodium thiosulfate standard solution (Sigma-Aldrich, Germany)
4. Diethyl ether (Sigma-Aldrich, Germany)
5. Ethyl alcohol (Sigma-Aldrich, USA)
6. Phenolphthalein (Sigma-Aldrich, USA)
7. Acetic acid (Sigma-Aldrich, USA)

8. Acetonitrile (RCI labscan, Thailand)
9. Sodium hydroxide (Sigma-Aldrich, USA)

Apparatus

1. Mixer (Cleo, Model CCB-404, Thailand)
2. Centrifuge (Retsch, Model D-422759, Germany)
3. Hot air oven (Lab Tech Model LDO-150F, South Korea)
4. Stereo Compound Microscope (Olympus BX 40, Japan)
5. Spectrophotometer (HACH, Model DR/4000U, USA)
6. Spray dryer (Labcono, Model S/N 2650, USA)
7. pH meter (Sartorius, Model Docu pH, Canada)
8. Hunter Lab Colorflex (Hunter Lab, Model 4510, USA)
9. Pump (Waston Marlow, Model 520 S/R)
10. Water activity analyser (NOVASINA, Model AW-CENTER 200)
11. Moisture analyser (SARTORIUS, Model MA 40)
12. Homogenizer (NIHONSEIKI KAISHA, Model AM-10, Japan)
13. Incubator (Shellab, Model 2005, USA)
14. Incubator (Shellab, Model 1535, USA)
15. Viscometer (RVDV II, Model 845X, USA)
16. Analytical balance with 4 decimal points (Sartorius, Model BSA224S AW, Germany)

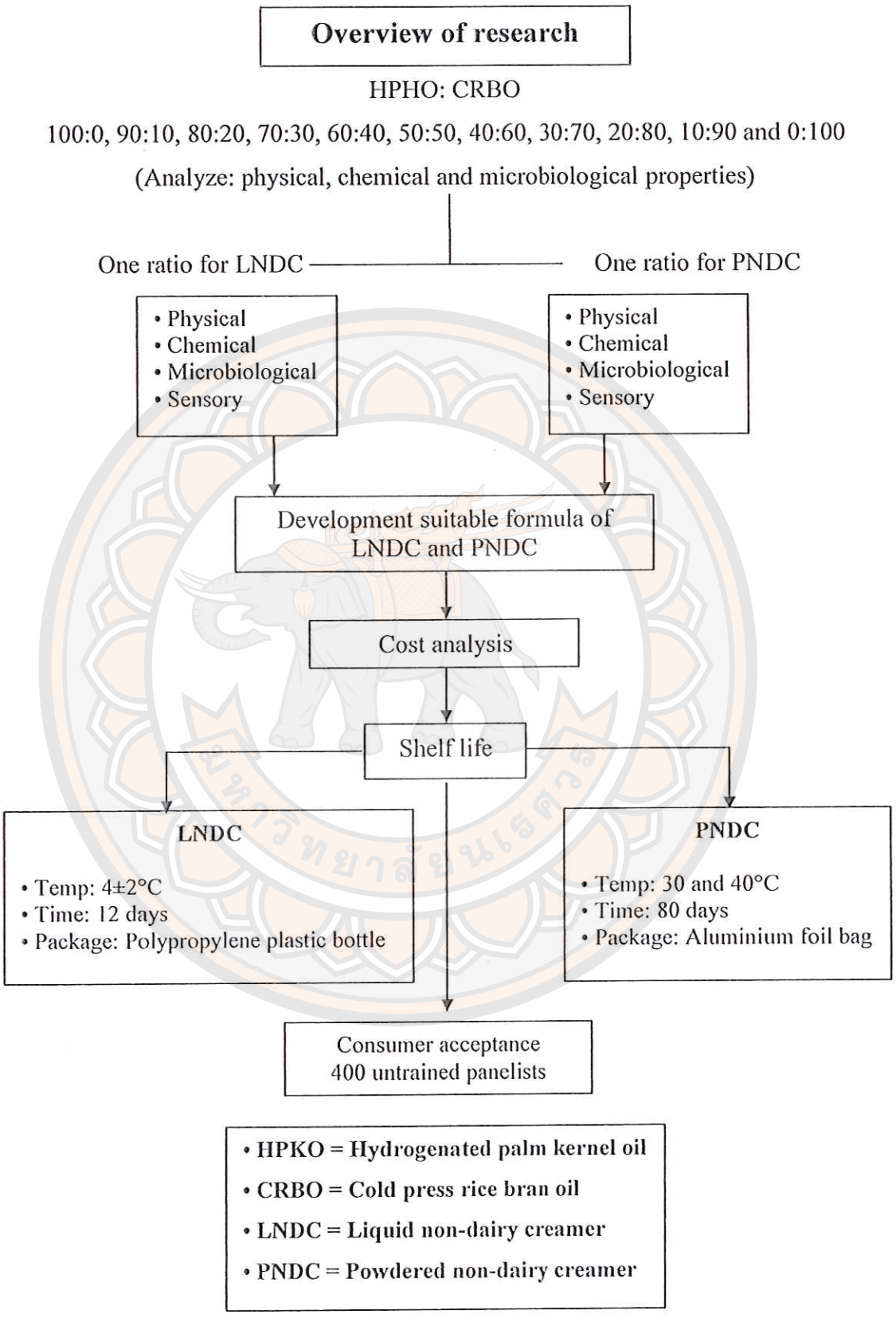

Figure 8 Overview of research

Part 1 Properties of hydrogenated palm kernel oil, cold press rice bran oil and their mixtures

In this research, there were 2 types of fat and oil used; HPKO and CRBO. For this part, it was aimed to determine the properties of HPKO, CRBO and their mixtures before using as a main ingredient for making LNDC and PNDC as to find out whether which mixing ratio of HPKO and CRBO would be appropriate for each product. In other words, it is to find out how much CRBO can be substituted HPKO for production of LNDC and PNDC.

The mixing ratio between HPKO and CRBO at the ratio of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100 (control) were studied. All treatments were determined for their physical, chemical and microbiological properties as following:

1. Physical properties of mixed oil (Method of determination was following Appendix F)

1.1 Viscosity (Brookfield)

1.2 Color (Hunter lab)

1.3 Absorbance (UV-VIS spectrophotometry)

2. Chemical properties of mixed oil (Method of determination was following Appendix G)

2.1 Fatty acids profile (In house method based on Compendium of Methods for Food Analysis. Thailand, 2003)

2.2 Antioxidative compositions

2.2.1 Vitamin E (α-Tocopherol) content (In house method based on Journal of Chromatography A, 1991, 825, 127-133)

2.2.2 γ-Oryzanol content (In house method based on ASEAN Food Journal, 2008, 15(1), 89-96)

2.2.3 Antioxidant activity (DPPH) (Anagnostopoulou, et al., 2006)

2.3 Trans fat content (In house method based on Compendium of Methods for Food Analysis. Thailand, 2003)

3. Microbiological properties of mixed oil (Method of determination was following Appendix H)

3.1 Total plate count (Maturin and Peeler, 2001)

3.2 Yeast and mold (Tournas, et al., 2001)

3.3 Coliform (Feng, Weagent and Grant, 2002)

3.4 *Escherichia coli* (Feng, Weagent and Grant, 2002)

Experimental design: The experimental design used was Complete Randomized Design (CRD) with 3 replications.

Part 2 Development of formula and process for liquid and powder non-dairy creamer from mixture of hydrogenated palm kernel oil, cold pressed rice bran oil and cost analysis

This part aimed to find out the suitable mixing ratio between HPKO and CRBO in processing to LNDC and PNDC by developing both formula and process. The suitable mixing ratio between HPKO and CRBO was selected. The standard formula and process of producing LNDC and PNDC from mixed HPKO and CRBO followed the methods of Singanusong and Noitup (2009). As there were possibilities of RBO to be replaced or substituted HPKO for production of LNDC and PNDC, it was possible to adjust the formula and process for each product in order to get high quality final products.

For cost analysis, it was aimed to find out whether it is financially possibly to manufacture LNDC and PNDC from mixed HPKO and CRBO.

The selected LNDC and PNDC from mixed HPKO and CRBO were analyzed for production cost in a laboratory scale.

The products produced in this section were 2 types:

1. Liquid non-dairy from mixed HPKO and CRBO
2. Powdered non-dairy from mixed HPKO and CRBO

1. Liquid non-dairy from mixed HPKO and CRBO

The 11 mixed oils (in part 1) were used to produce LNDC. There were mainly used in substitution of CRBO in the standard formula of LNDC (Table 10). The ingredients of LNDC were shown in Figure 8. The ingredients were mixed well

by using homogenizer at 5,000 rpm (Figure 10). The production process of LNDC was shown in Figure 11.

Table 10 Standard formula of LNDC

Ingredients	Amount (%)
Water	36.64
CRBO	36.29
Glucose syrup	21.45
Sodium caseinate	4.07
Maltodextrin (DE 10)	1.43
Potassium phosphate	0.10

Source: Singanusong and Noitup, 2009

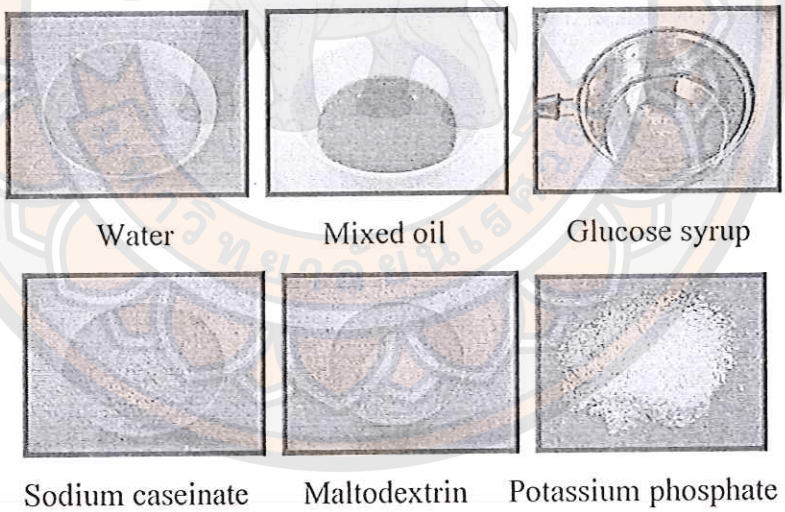

Figure 9 The ingredients of LNDC

Figure 10 The homogenizer used for production of LNDC

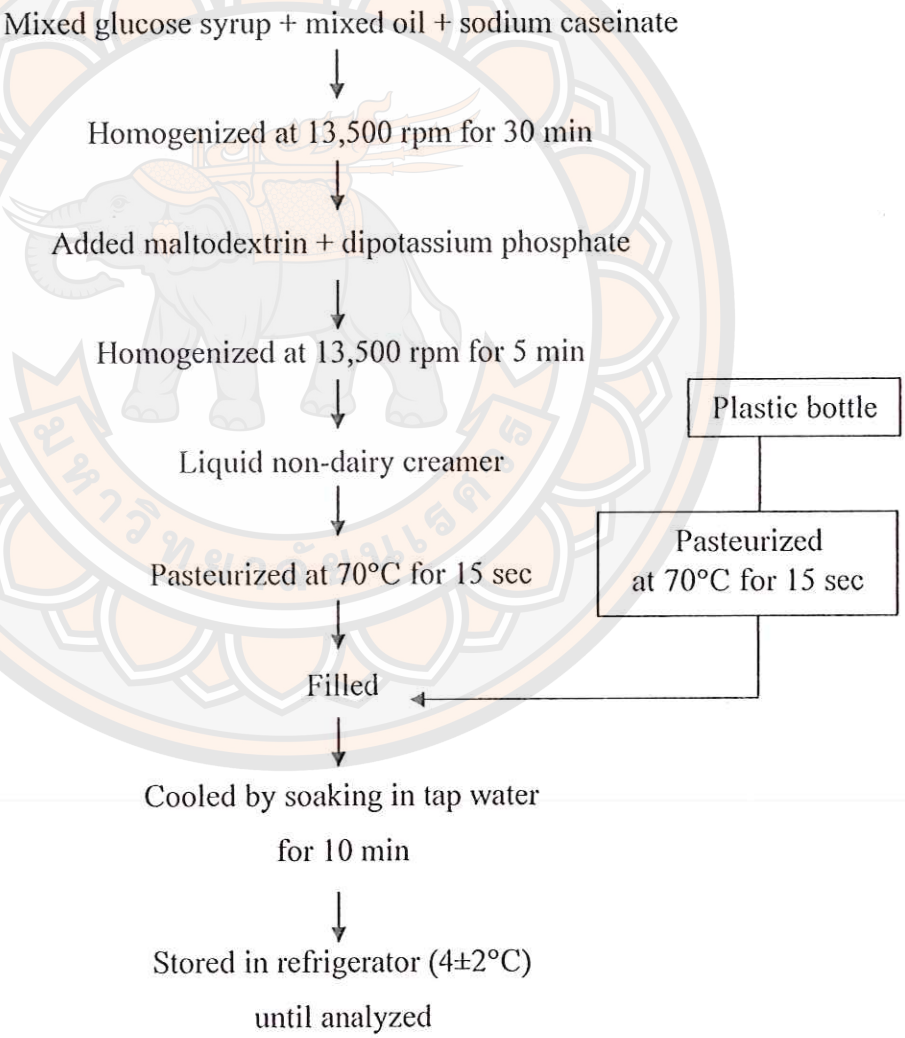

Figure 11 Flow chat of production of LNDC

Source: Singanusong and Noitup, 2009

2. Powdered non-dairy from mixed HPKO and CRBO

The 11 mixed oils (in part 1) were used to produce PNDC. They were substituted CRBO in the standard formula of PNDC (Table 11). The ingredients of PNDC were shown in Figure 12. The ingredients were mixed in homogenizer and transferred to spray dryer (Figure 13). The production process of PNDC was shown in Figure 14.

Table 11 Standard formula of PNDC

Ingredients	Amount (%)
Water	45.30
CRBO	16.30
Glucose syrup	25.40
Sodium caseinate	4.07
Skim milk powder	5.60
Lecithin	5.00
Maltodextrin (DE 10)	1.43

Source: Singanusong and Noitup, 2009

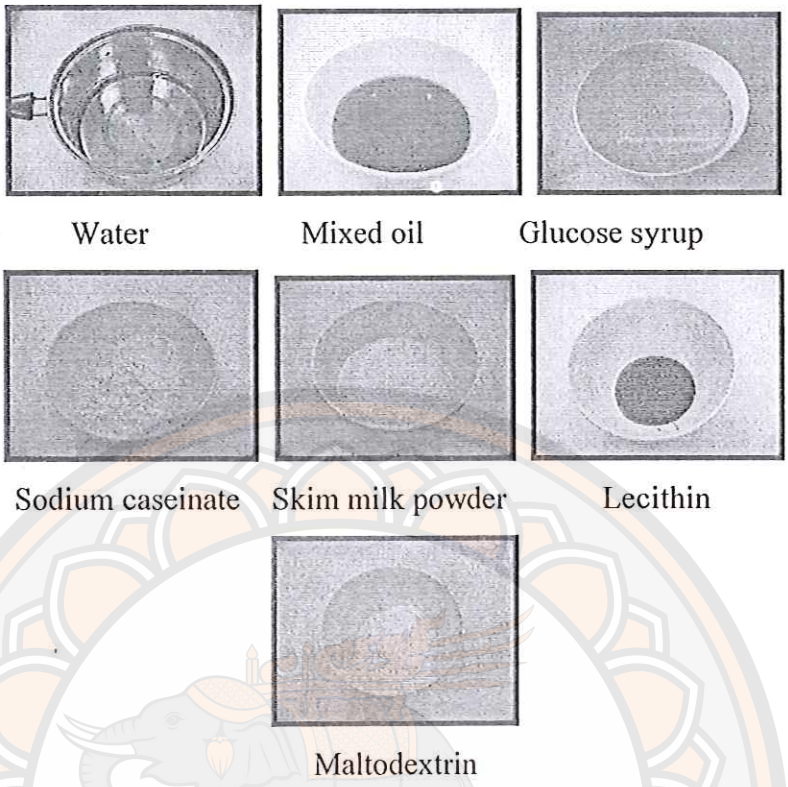

Figure 12 The ingredients of PNDC

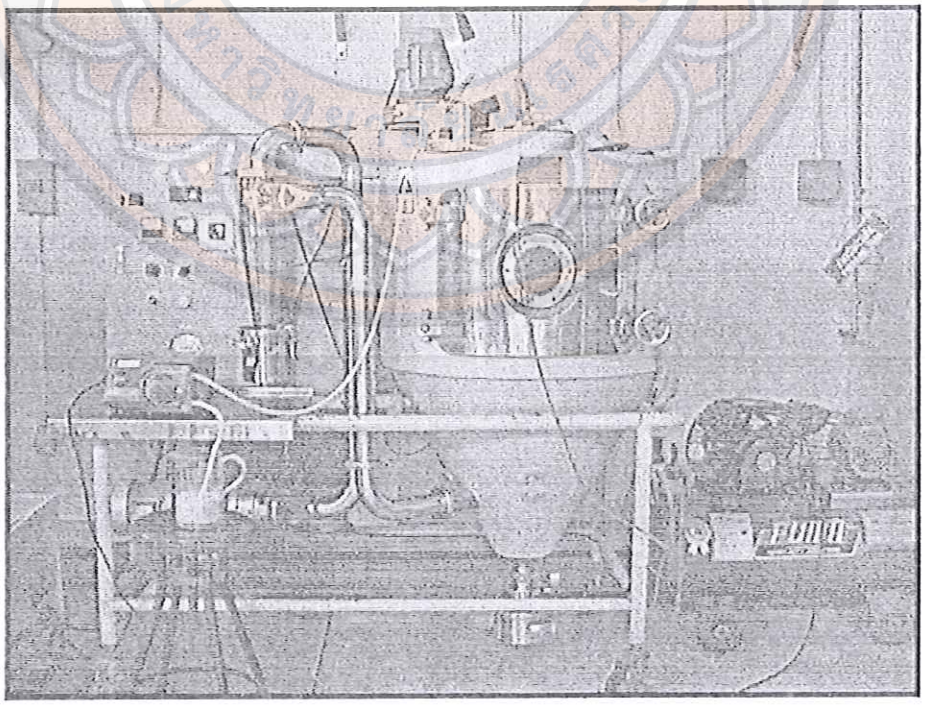

Figure 13 The spray drier used for the production of PNDC

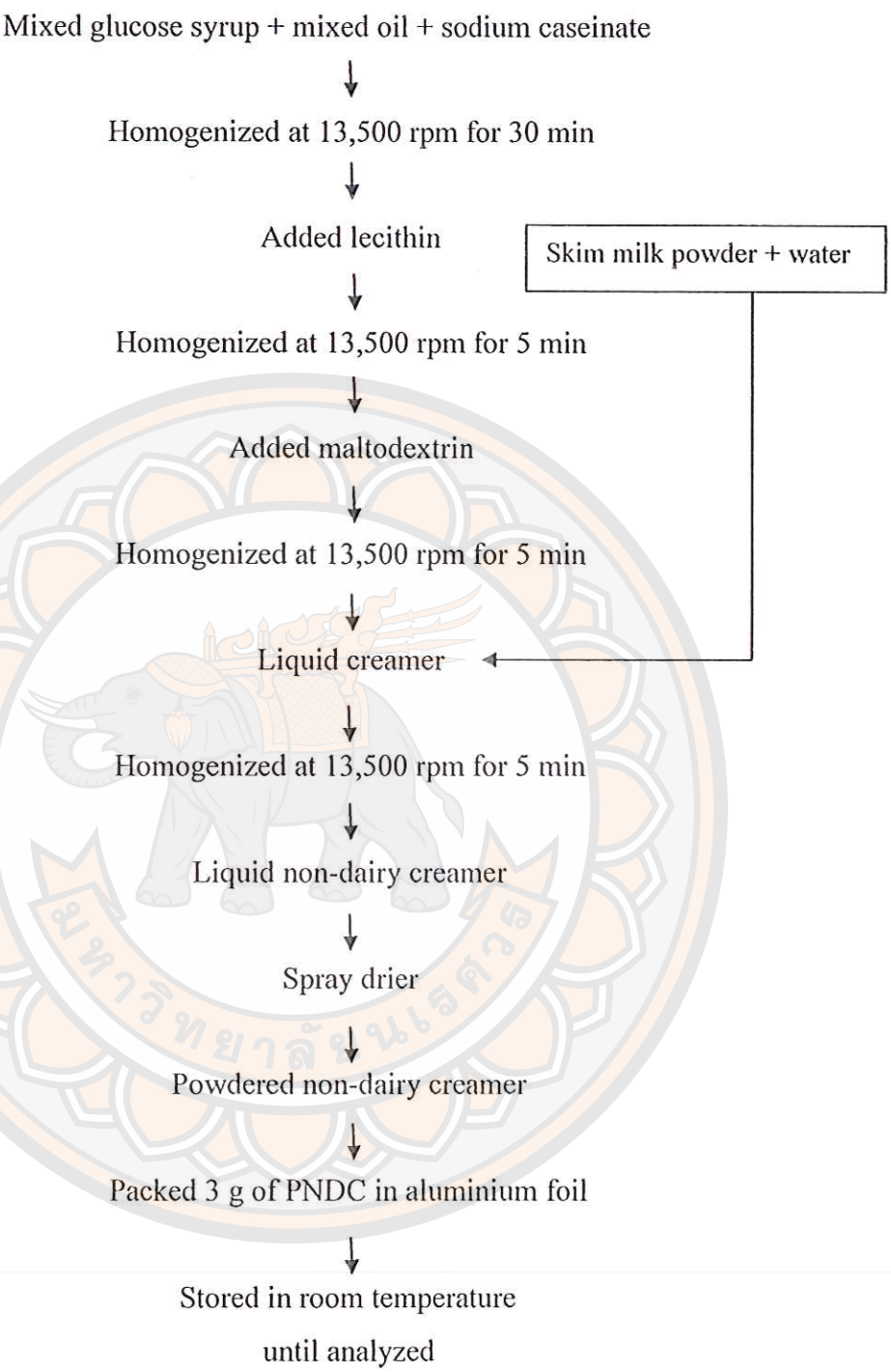

Figure 14 Flow chat of production of PNDC

Source: Singanusong and Noitup, 2009

The LNDC and PNDC from mixed HPKO and CRBO were examined for their properties as followings:

1. Physical properties of 11 formulas LNDC and PNDC (Method of determination was following Appendix F)

1.1 Color (Hunter lab)

1.2 Solubility (Al–khatani and Hassan, 1990)

1.3 Viscosity (Brookfield)

1.4 Bulk density (Nezbed and Zamrow, 1973)

2. Chemical properties of 11 formulas LNDC and PNDC (Method of determination was following Appendix G)

2.1 Fat content (AOAC, 1999)

2.2 Moisture content (Kha, Nguyen and Roach, 2010)

2.3 Water activity (Kha, Nguyen and Roach, 2010)

2.4 Acidity (as lactic acid) (Tseng and Zhao, 2013)

2.5 Antioxidant activity (DPPH) (Anagnostopoulou, et al., 2006)

2.6 Acid value (AOCS, 1995)

2.7 Peroxide value (Wrolstad, et al., 2005)

3. Microbiological properties of 11 formulas of LNDC and PNDC (Method of determination was following Appendix H)

3.1 Total plate count (Maturin and Peeler, 2001)

3.2 Yeast and mold (Tournas, et al., 2001)

3.3 Coliform (Feng, Weagent and Grant, 2002)

3.4 *Escherichia coli* (Feng, Weagent and Grant, 2002)

Experimental design: The experimental design used was Complete Randomized Design (CRD) with 3 replications.

4. Sensory properties of 11 formulas of LNDC and PNDC

All samples were sensory evaluated in term of color, odor, dispersibility, texture, taste and overall liking (Table 12) by 50 untrained panelists using 9-points hedonic scale where 1 = dislike extremely and 9 = like extremely.

Table 12 Evaluated attributes of LNDC and PNDC

Evaluated attributes	
LNDC	PNDC
Color of LNDC	Color of PNDC
Liquid suspension	Powder suspension
Odor of LNDC	Odor of PNDC
Color of coffee	Color of coffee
Texture of coffee	Texture of coffee
Taste of coffee	Taste of coffee
Overall liking	Dispersibility
	Overall liking

Experimental design: The experimental design for the sensory evaluation was Randomized Completely Block Design (RCBD).

Mixture design was necessary for the development of LNDC and PNDC because the surface of coffee had scum present. In this step, one ratio of mixed oil was selected for development of LNDC and PNDC by using mixture design. The selection criteria for consideration of the suitable formula were trans fat content and sensory evaluation.

Part 3 The shelf life of liquid and powder non-dairy creamer from mixture of hydrogenated palm kernel oil, cold press rice bran oil

This part aimed to find out the quality stability of the selected LNDC and PNDC from mixed HPKO and CRBO during storage.

The selected LNDC and PNDC from mixed HPKO and CRBO were packed in a normal package as the commercial ones and kept at refrigerated (4±2°C) for 14 days for LNDC and accelerated shelf life (30 and 40°C for 80 days) for PNDC. The LNDC were randomly examined every 3 days whereas the PNDC were randomly tested every 10 days. The properties to be tested were as following:

1. Quality properties of LNDC and PNDC during storage time

1.1 Physical properties of LNDC and PNDC during storage time (Method of determination was following Appendix F)

1.1.1 Color (Hunter lab)

1.1.2 Solubility (Al–khatani and Hassan, 1990)

1.1.3 Viscosity (Brookfield)

1.1.4 Bulk density (Nezbed and Zamrow, 1973)

1.2 Chemical properties of LNDC and PNDC during storage time (Method of determination was following Appendix G)

1.2.1 Moisture content (Kha, Nguyen and Roach, 2010)

1.2.2 Fat content (AOAC, 1999)

1.2.3 Water activity (Kha, Nguyen and Roach, 2010)

1.2.4 Antioxidant activity (DPPH) (Anagnostopoulou, et al., 2006)

1.2.5 Acidity (as lactic acid) (Tseng and Zhao, 2013)

1.2.6 Free fatty acid value (Wrolstad, et al., 2005)

1.2.7 Acid value (AOCS, 1995)

1.2.8 Peroxide value (Wrolstad, et al., 2005)

1.2.9 Vitamin E (α-Tocopherol) (In house method based on Journal of Chromatography A, 1991)

1.2.10 γ-Oryzanol content (In house method based on ASEAN Food Journal, 2008)

1.2.11 Trans fat content (In house method based on Compendium of Methods for Food Analysis. Thailand, 2003)

1.2.12 Fatty acid profile (In house method based on Compendium of Methods for Food Analysis. Thailand, 2003)

1.3 Microbiological properties of LNDC and PNDC during storage time (Method of determination was following Appendix H)

1.3.1 Total plate count (Maturin and Peeler, 2001)

1.3.2 Yeast and mold (Tournas, et al., 2001)

1.3.3 Coliform (Feng, Weagent and Grant, 2002)

1.3.4 *Escherichia coli* (Feng, Weagent and Grant, 2002)

Experimental design: The experimental design used was Complete Randomized Design (CRD) with 3 replications.

1.4 Sensory properties of LNDC and PNDC during storage time

The sensory attributes of LNDC and PNDC from mixed HPKO and CRBO were evaluated including color, odor, dispersibility, texture, taste and overall liking (Table 13) by 50 untrained panelists using the 9-points Hedonic scale.

Table 13 Evaluated attributes of LNDC and PNDC during storage time

Evaluated attributes	
LNDC	PNDC
Color of LNDC	Color of PNDC
Liquid suspension	Powder suspension
Rancidity	Rancidity
Odor of LNDC	Odor of PNDC
Color of coffee	Color of coffee
Texture of coffee	Texture of coffee
Taste of coffee	Taste of coffee
Overall liking	Dispersibility
	Overall liking

Experimental design: The experimental design for the sensory evaluation was Randomized Completely Block Design (RCBD).

Part 4 Marketing and consumer acceptance

This part aimed to find out whether the selected LNDC and PNDC from mixed HPKO and CRBO were accepted by the general consumers in terms of quality and price.

The selected LNDC and PNDC from mixed HPKO and CRBO were sensory tasted by 400 untrained consumers using the 9-point Hedonic scale using the Central Location Test at Naresuan University. The collected details of consumers included

sex, education, career, frequency of eating the products, willingness to purchase the products, etc.

The questionnaire composed of 4 sections as following:

Section 1: General question about consumer

Section 2: Information about consumer behavior in non-dairy creamer

Section 3: Information about product development

Section 4: Characteristic about non-dairy creamer in consumer need

Section 1. General question about consumer

1. Gender

- ☐ Male
- ☐ Female

2. Age

- ☐ Less than 20 years old
- ☐ 20-25 years old
- ☐ 26- 35 years old
- ☐ 36- 45 years old
- ☐ 46- 55 years old
- ☐ More than 56 years old

3. Occupation

- ☐ School student
- ☐ College student
- ☐ Government officer
- ☐ Private employee
- ☐ State enterprise employee
- ☐ Agriculturist
- ☐ Laborer
- ☐ Housekeeper
- ☐ Others

4. Salary

- ☐ Less than 6,000 Baht/month
- ☐ 6,001- 9,000 Baht/month
- ☐ 9,001-12,000 Baht/month
- ☐ 12,001-15,000 Baht/month
- ☐ 15,001-18,000 Baht/month
- ☐ More than 18,000 Baht/month

5. Living style

- ☐ Single
- ☐ Family
- ☐ Co-others

6. Education

- ☐ Primary school
- ☐ Secondary school
- ☐ High school
- ☐ Diploma
- ☐ Bachelor
- ☐ Master or Ph.D.

Section 2: Information about consumer behavior in non-dairy creamer

7. Consume and never consume the product

- ☐ Consume
- ☐ Never consume

8. Like and dislike the product

- ☐ Like
- ☐ Dislike

9. Reason for consumption of the product

- ☐ Taste
- ☐ Texture
- ☐ Coffee taste
- ☐ Odor
- ☐ Nutritional value
- ☐ Others

10. Reason for not consumption of the product

- ☐ Taste
- ☐ Expensive
- ☐ Odor
- ☐ Texture
- ☐ Others

11. Frequency of consumption

- ☐ 1 time/day
- ☐ 2 times/day
- ☐ 3 times/day
- ☐ More than 3 times/day
- ☐ 1 time/week
- ☐ 1 time/month
- ☐ 2-3 times/month
- ☐ Seldom

12. Use the product in

- ☐ Tea
- ☐ Coffee
- ☐ Cocoa

13. Place for buying the product

- ☐ Department store (Big C, Lotus, Makro)
- ☐ Convenient shop (7-11, 108 Shop)
- ☐ Grocery

14. Size of the product normally buy

- ☐ 1 g
- ☐ 5 g
- ☐ 10 g
- ☐ 15 g
- ☐ 20 g

Section 3: Information about product development

15. Do you know this product?

- ☐ Known
- ☐ Unknown

16. Have you ever consumed the product?

- ☐ Yes
- ☐ Never

17. If available, are you interested to buy this product?

- ☐ Interest
- ☐ No interest

18. Why are you interested to buy this product?

- ☐ New product
- ☐ Nutritional value
- ☐ Price
- ☐ Regular consume

19. Why are you not interested to buy this product?

- ☐ Scare
- ☐ Not drink tea or coffee
- ☐ Others

Section 4: Characteristic about non-dairy creamer in consumer need

20. The most important properties of the product

- ☐ Taste
- ☐ Nutritional value
- ☐ Odor
- ☐ Color / appearance
- ☐ Texture
- ☐ Others

21. Prefered type of packaging for liquid non-dairy creamer

- ☐ Plastic bag
- ☐ Plastic cup
- ☐ Glass bottle
- ☐ Aluminum foil
- ☐ Others

22. Preferred type of packaging for powdered non-dairy creamer

- ☐ Plastic bag
- ☐ Plastic cup
- ☐ Glass bottle
- ☐ Aluminum foil
- ☐ Others

23. Appropriated serving size for liquid non-dairy creamer

- ☐ 15 ml/ pack in plastic cup
- ☐ 20 ml/ pack in plastic cup
- ☐ 100 ml/ pack in plastic bottle
- ☐ 200 ml/ pack in plastic bottle
- ☐ 100 ml/ pack in glass bottle
- ☐ 200 ml/ pack in glass bottle
- ☐ Others

24. Appropriated serving size for powdered non-dairy creamer

- ☐ 3 g/50 packs in plastic bag
- ☐ 3 g/100 packs in plastic bag
- ☐ 75 g/ pack in aluminum foil
- ☐ 100 g/ pack in aluminum foil
- ☐ 200 g/ pack in aluminum foil
- ☐ 400 g/ pack in plastic bottle
- ☐ Others

25. Appropriated price you prefer for liquid non-dairy creamer (referred 23)

- ☐ 1-5 Baht
- ☐ 6-10 Baht
- ☐ 11-30 Baht
- ☐ 31-60 Baht
- ☐ 61-90 Baht
- ☐ 91-120 Baht
- ☐ Others

26. Appropriated price you prefer in powdered non-dairy creamer (referred 24)

- ☐ 15-30 Baht
- ☐ 31-50 Baht
- ☐ 51-60 Baht
- ☐ 61-70 Baht
- ☐ 71-80 Baht
- ☐ Others

27. The most important factor for buying non-dairy creamer

- ☐ Price
- ☐ Nutritional value
- ☐ Type of packaging
- ☐ Amount per unit
- ☐ Selling place
- ☐ Convenient
- ☐ Others

5. Data analysis

The data from all experimental parts were subjected to analysis of variance (ANOVA). The significant difference of means was analyzed using the Duncan's New Multiple Range Test (DMRT). The experiment was conducted in three replications (except part 4).

CHAPTER IV

RESULTS AND DISCUSSION

This chapter showed the results and discussion of this study which were divided into 4 parts. Part 1 showed the properties of HPKO, CRBO and their mixtures. Part 2 illustrated development of formula and process for LNDC and PNDC from mixture of HPKO and CRBO and cost analysis. Part 3 showed the shelf life of LNDC and PNDC from mixture of HPKO and CRBO. Part 4 demonstrated marketing and consumer acceptance of LNDC and PNDC.

Part 1 Properties of hydrogenated palm kernel oil, cold pressed rice bran oil and their mixtures

The mixing ratio between HPKO and CRBO at 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100 (control) were studied. All treatments were determined for their physical, chemical and microbiological properties as following:

1. Physical properties of mixed oil

Color, viscosity and absorbance of HPKO, CRBO and their mixtures

The properties of CRBO were determined after degumming step. Degumming is to remove natural gums presented in the oil which could hinder the mixtures for further utilization in food products (Singanusong and Noitup, 2009). The color and viscosity of HPKO, CRBO and their mixtures were shown in Table 14. The appearance of mixed oil was shown in Figure 14 as the color of HPKO (A) and CRBO (K) at the ratio of 90:10 (B), 80:20 (C), 70:30 (D), 60:40 (E), 50:50 (F), 40:60 (G), 30:70 (H), 20:80 (I) and 10:90 (J). The mixed oil became darker when mixed with high ratio of CRBO.

Table 14 Viscosity and color of HPKO, CRBO and their mixtures

HPKO:CRBO	Color			Viscosity (cp.) at 100 rpm
	L*	a*	b*	
100:0	$89.82^{a}\pm0.01$	$-1.78^{k}\pm0.05$	$39.83^{k}\pm0.02$	$46^{c}\pm1.14$
90:10	$83.14^{b}\pm0.03$	$-4.12^{j}\pm0.02$	$48.48^{j}\pm0.04$	$46^{c}\pm1.75$
80:20	$77.87^{c}\pm0.12$	$0.78^{i}\pm0.01$	$52.14^{i}\pm0.01$	$46^{c}\pm1.10$
70:30	$72.82^{d}\pm0.02$	$3.71^{h}\pm0.01$	$60.47^{h}\pm0.02$	$46^{c}\pm1.36$
60:40	$69.09^{e}\pm0.13$	$6.99^{g}\pm0.01$	$66.32^{g}\pm0.01$	$46^{c}\pm1.05$
50:50	$65.40^{f}\pm0.30$	$10.06^{f}\pm0.02$	$71.09^{f}\pm0.02$	$50^{b}\pm1.30$
40:60	$62.21^{g}\pm0.30$	$12.55^{e}\pm0.01$	$78.70^{e}\pm0.03$	$50^{b}\pm1.20$
30:70	$59.11^{h}\pm0.31$	$14.40^{d}\pm0.03$	$82.34^{d}\pm0.01$	$50^{b}\pm1.52$
20:80	$56.11^{i}\pm0.12$	$15.78^{c}\pm0.05$	$86.59^{c}\pm0.04$	$54^{a}\pm1.25$
10:90	$53.85^{j}\pm0.25$	$16.88^{b}\pm0.01$	$90.41^{b}\pm0.02$	$54^{a}\pm1.10$
0:100	$48.16^{k}\pm0.30$	$20.12^{a}\pm0.01$	$97.60^{a}\pm0.01$	$54^{a}\pm1.24$

Note: Means in the column with different superscripts were significantly different ($P\leq0.05$)

The viscosities of the HPKO: CRBO mixtures of 100:0, 90:10, 80:20, 70:30 and 60:40 were 46 cp. ($p>0.05$) whereas the HPKO: CRBO mixtures of 50:50, 40:60 and 30:70 were 50 cp. ($p>0.05$). The samples with mixing ratios of HPKO: RBO at 20:80, 10:90 and 0:100 had the highest viscosity (54 cp.) at 100 rpm ($p\leq0.05$) but not significantly different ($p>0.05$) from each other. The viscosity of the mixtures and even the pure CRBO in this study was much higher than that found in RBO (78.60 cp.) reported by Jennings and Akoh (2010). This might be due to differences in genetic and environmental factors, extraction and measurement conditions.

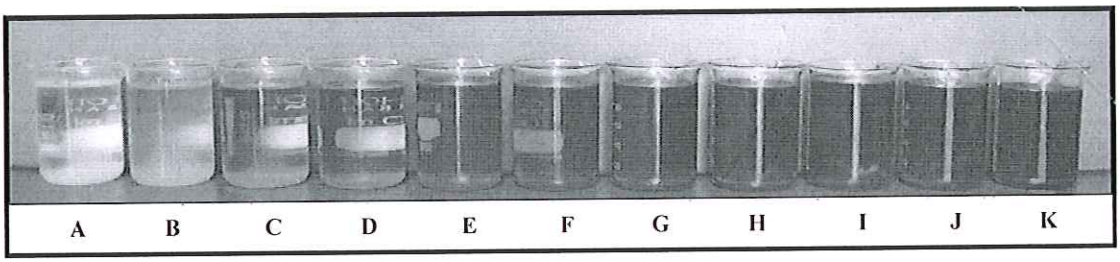

Figure 15 The appearance of HPKO (A), CRBO (K) and their mixtures (B-J)

HPKO was transparent light yellow while CRBO was dark brown in color. When compared the color of HPKO and CRBO to the Munsell Book of Color, it was found that level of color and brightness/intensity of HPKO were 5Y and 9/2, respectively and for CRBO were 10R and 2.5/2, respectively. The lightness (L*) of pure HPKO (89.82) was higher than that of pure CRBO (48.16) ($p \leq 0.05$) and that of HPKO (85.77) reported by Azlan, et al. (2010). However, the L* of CRBO (48.16) in this present study was much higher than the values reported by Jennings and Akoh (2009) and Thanonkaew, et al. (2012) which were 34.50 and 10.44, respectively. Due mainly to its dark brown in color, as the ratio of CRBO increased, the L* significantly decreased while the redness (a*) and yellowness (b*) increased ($p \leq 0.05$). Figure 16 showed the absorbance of HPKO and CRBO within the visible wavelength of 380-750 nm.

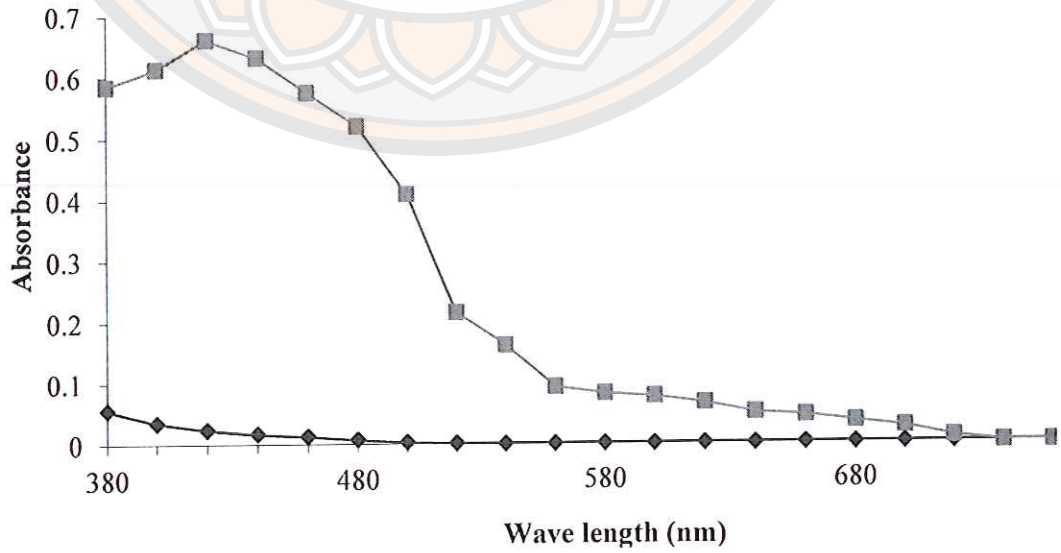

Figure 16 Visible absorption spectrum of HPKO (◆) and CRBO (■)

Compared with CRBO, HPKO had significantly lower absorbance ($p \leq 0.05$). As 420 nm offered the highest absorbance, therefore, it was selected to further used in absorbance reading for the mixtures between HPKO: CRBO and the results were shown in Figure 17. The absorbance was significantly increased with increasing CRBO ratio, which was in agreement with the results of L*.

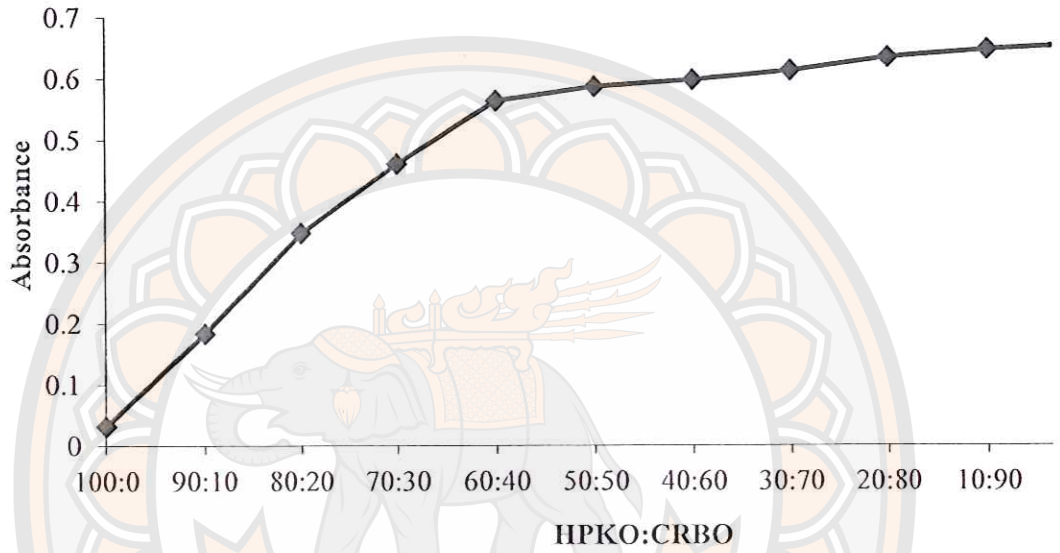

Figure 17 Visible absorption spectrum (420 nm) of HPKO, CRBO and their mixtures

2. Chemical properties

Fatty Acids Profile

The 10 fatty acids (FA) present in HPKO in descending order were lauric, stearic, myristic, palmitic, capric, caprylic, elaidic, oleic, arachidic and caproic acids. In contrast, the 5 FA found in CRBO in descending order were oleic, linoleic, palmitic, stearic and linolenic acids (Tables 15 and 16), similar to those reported by Singanusong (2012). The composition of saturated fatty acid (SFA) and unsaturated fatty acid (UFA) of HPKO, CRBO and their mixtures were illustrated in Tables 15 and 16, respectively.

Table 15 Composition of saturated fatty acids of HPKO, CRBO and their mixtures

HPKO: CRBO	Content (g/100 g)								Total (g/100 g)
	Caproic (C6:0)	Caprylic (C8:0)	Capric (C10:0)	Lauric (C12:0)	Myristic (C14:0)	Palmitic (C16:0)	Stearic (C18:0)	Arachidic (C20:0)	
100:0	0.13	3.52^{a}	3.57^{a}	45.48^{a}	12.01^{a}	6.86^{k}	19.80^{a}	0.15	91.52^{a}
90:10	-	2.19^{d}	3.40^{b}	43.75^{b}	11.88^{b}	8.94^{j}	14.61^{b}	-	84.77^{b}
80:20	-	3.20^{b}	3.28^{c}	39.07^{c}	10.86^{c}	10.11^{i}	13.93^{c}	-	80.45^{c}
70:30	-	2.28^{c}	2.70^{d}	33.55^{d}	9.57^{d}	11.64^{h}	13.83^{d}	-	73.57^{d}
60:40	-	1.07^{g}	2.19^{e}	29.59^{e}	8.69^{e}	13.39^{g}	11.98^{e}	-	66.91^{e}
50:50	-	1.53^{f}	2.01^{f}	26.45^{f}	7.51^{f}	14.48^{f}	9.75^{f}	-	61.73^{f}
40:60	-	1.81^{e}	1.92^{g}	23.28^{g}	6.54^{g}	16.24^{e}	7.63^{g}	-	57.42^{g}
30:70	-	0.86^{h}	1.17^{h}	15.12^{h}	4.63^{h}	17.59^{d}	7.17^{h}	-	46.54^{h}
20:80	-	-	0.62^{i}	9.57^{i}	3.44^{i}	19.99^{c}	5.72^{i}	-	39.34^{i}
10:90	-	-	0.42^{j}	5.47^{j}	2.06^{j}	21.58^{a}	3.54^{j}	-	33.07^{j}
0:100	-	-	-	-	-	21.00^{b}	2.50^{k}	-	23.50^{k}

Note: Means in the column with different superscripts were significantly different ($P \leq 0.05$)

The SFA composition of HPKO, CRBO and their mixtures was shown in Table 15. HPKO consisted of short-chain (6:0-10:0), medium-chain (12:0-14:0) and long-chain FA (16:0-20:0) but CRBO had only long-chain FA (16:0-18:0). These findings were consistent to that of Rattanapanone (2005) and Singanusong (2012). The lauric acid was predominant SFA in HPKO (45.48 g/100 g), therefore, the amount was slightly decreased with decreasing ratio of HPKO. On the other hand, palmitic acid was the major SFA found in CRBO (21.00 g/100 g), which was consistent to the findings of Chotimarkorn and Silalai (2008) (20.70 g/100 g) but higher than that reported by Singanusong (2012) (13.71 g/100 g). The total SFA decreased with increasing ratio of CRBO. In contrast, the total UFA increased with decreasing ratio of HPKO (Table 16).

The major UFA found in CRBO were oleic and linoleic acids; 44.30 and 30.80 g/100 g, respectively. The data were in agreement with findings of Chotimarkorn and Silalai (2008) who stated that oleic and linoleic acid contents in CRBO were 44.10

and 28.10 g/100 g, respectively. However, the values were higher than that reported by Singanusong (2012) who stated that oleic and linoleic acid contents in CRBO were 27.18 and 21.62 g/100 g, respectively. The differences could be due to differences in genetic and environmental factors and extraction methods.

Table 16 Composition of unsaturated fatty acids of HPKO, CRBO and their mixtures

HPKO:CRBO	Content (g/100 g)				
	Elaidic (C18:1, trans)	Oleic (C18:1, cis-9)	Linoleic (C18:2, cis)	Linolenic (C18:3)	Total (g/100 g)
100:0	2.61^{a}	1.45^{k}	-	-	4.06^{k}
90:10	1.93^{b}	5.92^{j}	2.98^{j}	-	10.83^{j}
80:20	1.85^{c}	8.31^{i}	4.98^{i}	-	15.14^{i}
70:30	-	13.82^{h}	8.21^{h}	-	22.03^{h}
60:40	-	17.41^{g}	11.27^{g}	-	28.68^{g}
50:50	-	20.11^{f}	13.76^{f}	-	33.87^{f}
40:60	-	22.14^{e}	16.03^{e}	-	38.17^{e}
30:70	-	28.18^{d}	20.86^{d}	-	49.04^{d}
20:80	-	32.07^{c}	24.20^{c}	-	56.27^{c}
10:90	-	35.31^{b}	27.21^{b}	-	62.52^{b}
0:100		44.30^{a}	30.80^{a}	1.4	76.50^{a}

Note: Means in the column with different superscripts were significantly different ($P \leq 0.05$)

Pure HPKO contained mainly SFA, a few monounsaturated fatty acids (MUFA) and no polyunsaturated fatty acids (PUFA) whereas pure CRBO contained mainly MUFA, following by PUFA and SFA (Table 17) which was in agreement with those reported by Singanusong (2012). The suitable Polyunsaturated: Monounsaturated: Saturated or PMS ratio recommended by World Health Organization, American Heart

Society, and Food and Agriculture Administration was 1: 1.4: 1 which is closely to the PMS ratio of CRBO (1.4: 1.9: 1).

Table 17 Unsaturated and saturated fatty acid contents and PMS ratio of HPKO, CRBO and their mixtures

HPKO:CRBO	Unsaturated fatty acids		Saturated fatty acids (g/100 g)	PMS ratio
	Polyunsaturated (g/100 g)	Monounsaturated (g/100 g)		
100:0	-	4.06^{k}	91.52^{a}	0:0.004:1
90:10	2.98^{j}	7.85^{j}	84.77^{b}	0.035:0.092:1
80:20	4.98^{i}	10.16^{i}	80.45^{c}	0.061:0.126:1
70:30	8.21^{h}	13.82^{h}	73.57^{d}	0.111:0.187:1
60:40	11.27^{g}	17.41^{g}	66.91^{e}	0.168:0.260:1
50:50	13.76^{f}	20.11^{f}	61.73^{f}	0.222:0.325:1
40:60	16.03^{e}	22.14^{e}	57.42^{g}	0.279:0.385:1
30:70	20.86^{d}	28.18^{d}	46.54^{h}	0.448:0.605:1
20:80	24.20^{c}	32.07^{c}	39.34^{i}	0.615:0.815:1
10:90	27.21^{b}	35.31^{b}	33.07^{j}	0.822:1.067:1
0:100	32.20^{a}	44.30^{a}	23.50^{k}	1.370:1.885:1

Note: Means in the column with different superscripts were significantly different ($P \leq 0.05$)

The increase in the ratio of CRBO would increase the levels of MUFA and PUFA but decrease the levels of SFA in the mixtures. This was also obviously evident by changing in their PMS ratio as shown in Table 17 and Figure 18.

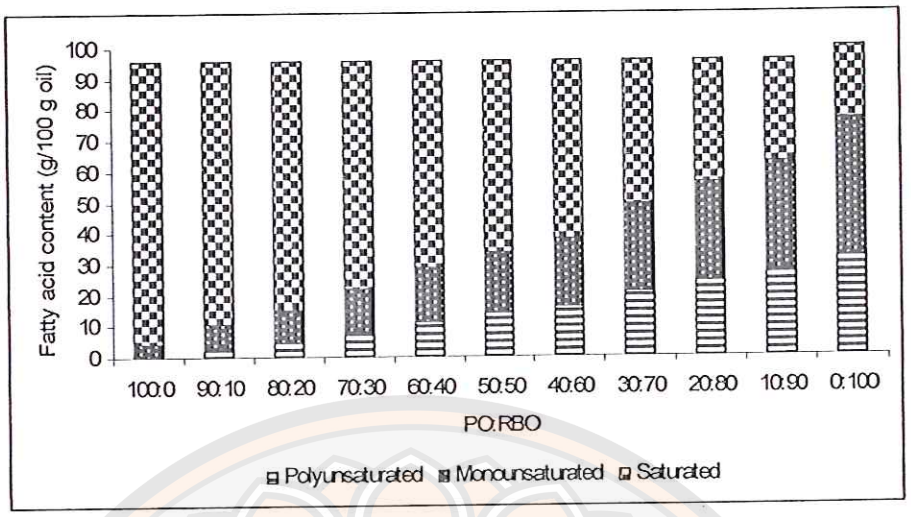

Figure 18 PMS ratio of HPKO, CRBO and their mixtures

Antioxidative Compositions

As shown in Table 18 and Figure 19a, γ-oryzanol was only found in CRBO, therefore, any mixtures that contained CRBO would have γ-oryzanol. Pure CRBO had significantly the highest γ-oryzanol content (1,155 mg/100 g oil) and antioxidant activity (94.24%) ($p \leq 0.05$) while pure HPKO had no γ-oryzanol and significantly the lowest antioxidant activity (9.26%) ($p \leq 0.05$). The γ-oryzanol content of CRBO was the same to the value reported by Singanusong (2012) but higher than the reported values of Thanonkaew, et al. (2012) (203.00 mg/100 g oil) and Chotimarkorn and Silalai (2008) (49.58 mg/100 g oil).

Table 18 γ-Oryzanol content, α-tocopherol content, antioxidant activity and trans fat content of HPKO, CRBO and their mixtures

HPKO:CRBO	γ-Oryzanol (mg/100g oil)	α-Tocopherol (mg/100g oil)	Antioxidant activity (DPPH) (%)	Trans fat (g/100g oil)
100:0	-	$<0.09^{h}$	$9.26^{k}\pm0.20$	2.73^{a}
90:10	36.38^{j}	$<0.09^{h}$	$20.86^{j}\pm0.33$	1.93^{b}
80:20	133.34^{i}	$<0.09^{h}$	$28.35^{i}\pm0.55$	1.85^{c}
70:30	191.79^{h}	$<0.09^{h}$	$36.16^{h}\pm0.25$	-
60:40	227.16^{g}	4.47^{g}	$42.79^{g}\pm0.32$	-
50:50	509.60^{f}	9.96^{f}	$51.92^{f}\pm0.60$	-
40:60	626.42^{e}	14.02^{e}	$59.41^{e}\pm0.22$	-
30:70	753.30^{d}	27.36^{d}	$65.32^{d}\pm0.10$	-
20:80	838.84^{c}	27.78^{e}	$74.80^{c}\pm0.15$	-
10:90	917.14^{b}	29.97^{b}	$86.02^{b}\pm0.40$	-
0:100	$1,155.00^{a}$	30.82^{a}	$94.24^{a}\pm0.35$	-

Note: Means in the column with different superscripts were significantly different ($P\leq0.05$)

The α-tocopherol content increased with increasing ratio of CRBO (Table 18 and Figure 18b), it was very low (0.09 mg/100 g oil) for HPKO but much higher (30.82 mg/100 g oil) for CRBO. The α-tocopherol content of HPKO was lower than the reported value of Azlan, et al. (2010) (51.91 mg/100 g oil), while the α-tocopherol content of CRBO was higher than the reported value of Chotimarkorn and Silalai (2008) (6.91 mg/100 g oil).

Pure HPKO had the lowest antioxidant activity while pure CRBO had the highest ($p\leq0.05$). The antioxidant activity increased significantly ($p\leq0.05$) with increasing the ratio of CRBO (Table 18 and Figure 19c).

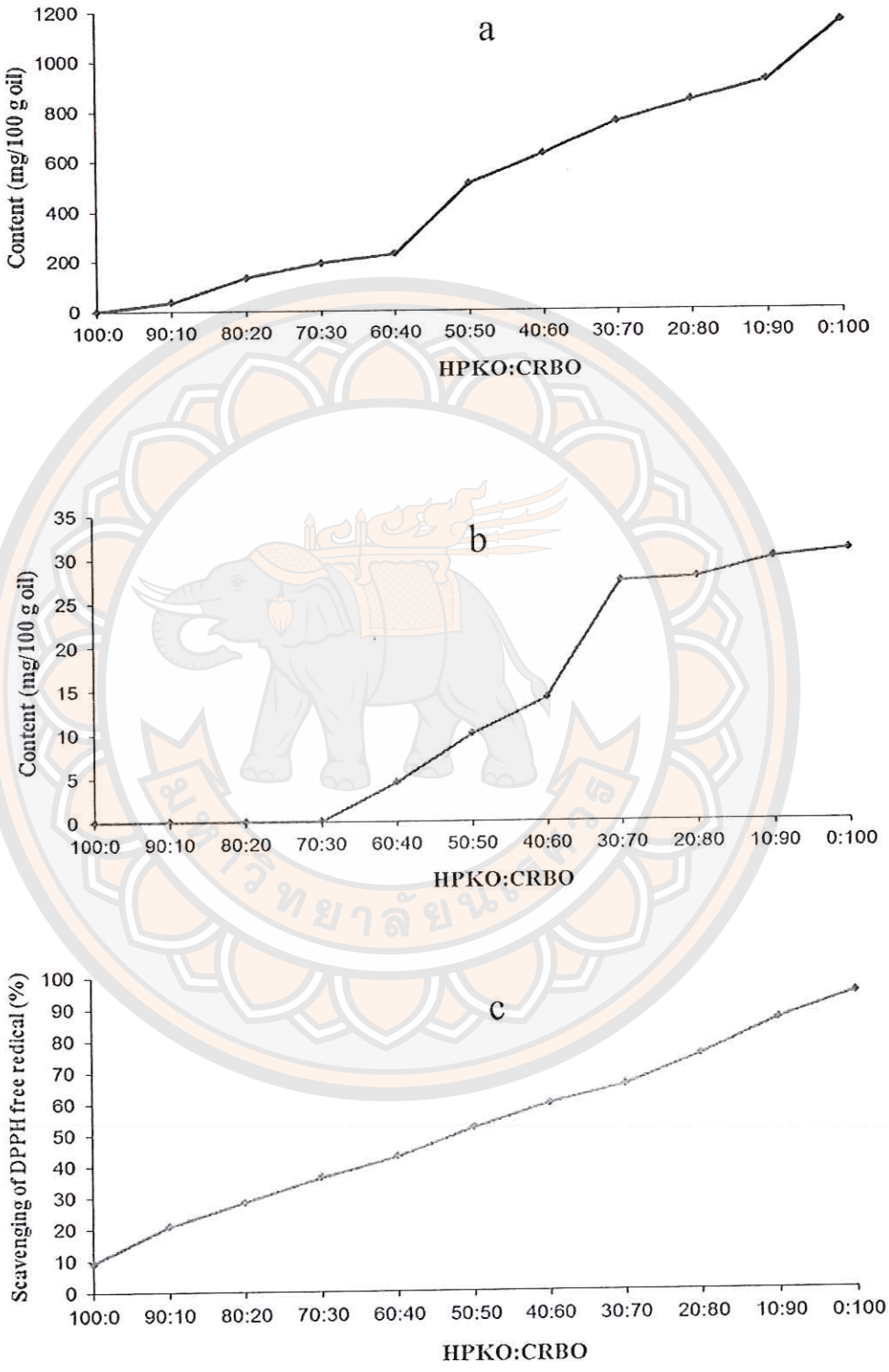

Figure 19 γ-Oryzanol (a), α-tocopherol (b) and antioxidant activity (c) of HPKO, CRBO and their mixtures

It could be obviously concluded that the high antioxidant activity of CRBO was mainly from γ-oryzanol as the trend lines of both γ-oryzanol (Figure 12a) and antioxidant activity (Figure 12c) increased steadily with increasing ratio of CRBO. In Figure 18a, pure HPKO had very low concentration of α-tocopherol and CRBO at 0-30% were too low to be detected. Substitution of HPKO by CRBO from 40% would provide α-tocopherol to the mixtures and hence antioxidant activity. Thanonkaew, et al. (2012) found that CRBO had antioxidant activity of 30% which was much lower than the value (94.24%) found in this present study. Genetic and environmental factors as well as extraction and analytical methods would contribute to these differences.

Trans Fat

Trans fat was only detected in the HPKO:CRBO mixtures of 100:0, 90:10 and 80:20 (Table 18), indicating that substitution of HPKO by CRBO from 30% would eliminate trans fat, hence the blended oils would offer no health risk from trans fat as it has been reported to be a contributor to heart disease and cancer (Jennings and Akoh, 2010).

3. Microorganisms

The total viable count, yeast and mold, coliform and *Escherichia coli* were not detectable in all samples (Table 19). Therefore, all the treatments of the blended oils were safe from microorganism contamination or growth.

Table 19 Microbiological counts of HPKO, CRBO and their mixtures

HPKO:CRBO	Total viable count (CFU/mL)^{ns}	Yeast and mold (CFU/mL)^{ns}	Coliform (MPN/mL)^{ns}	*Escherichia coli* (MPN/mL)^{ns}
100:0	<10	<10	<3	<3
90:10	<10	<10	<3	<3
80:20	<10	<10	<3	<3
70:30	<10	<10	<3	<3
60:40	<10	<10	<3	<3
50:50	<10	<10	<3	<3

Table 19 (cont.)

HPKO:CRBO	Total viable count (CFU/mL)^{ns}	Yeast and mold (CFU/mL)^{ns}	Coliform (MPN/mL)^{ns}	*Escherichia coli* (MPN/mL)^{ns}
40:60	<10	<10	<3	<3
30:70	<10	<10	<3	<3
20:80	<10	<10	<3	<3
10:90	<10	<10	<3	<3
0:100	<10	<10	<3	<3

Note: ^{ns} Means in the column were not significantly different (P>0.05)

HPKO, CRBO and their mixtures were further applied in part 2. Both LNDC and PNDC were developed for formula and process by changing amount of different oil ratio in the standard formula.

Part 2 Development of formula and process for liquid and powder non-dairy creamer from mixture of hydrogenated palm kernel oil, cold press rice bran oil and cost analysis

This part aimed to find out the suitable mixing ratio between HPKO and CRBO in processing of LNDC and PNDC by developing both formula and process. The suitable mixing ratio between HPKO and CRBO was selected. The standard formula and process of producing LNDC and PNDC from mixed HPKO and CRBO was followed the methods of Singanusong and Noitup (2009). As there were possibilities of CRBO to be replaced or substituted HPKO for production of LNDC and PNDC, it was possible to adjust the formula and process for each product in order to get high quality final products.

The selected LNDC and PNDC from mixed HPKO and CRBO was analyzed for production cost in a laboratory scale.

1. Properties of 11 formulas of LNDC

1.1 Physical properties of 11 formulas of LNDC

The appearance of the LNDC was shown in Figure 20. LNDC was white in color and had a few odor of rice bran. The rice bran odor was depending on ratio of CRBO. The LNDC of 100% of CRBO (0:100) had stronger rice bran odor than other ratios. Rice bran odor was distinct since CRBO used in this study did not pass through the odorization process which eliminated the rice bran odor.

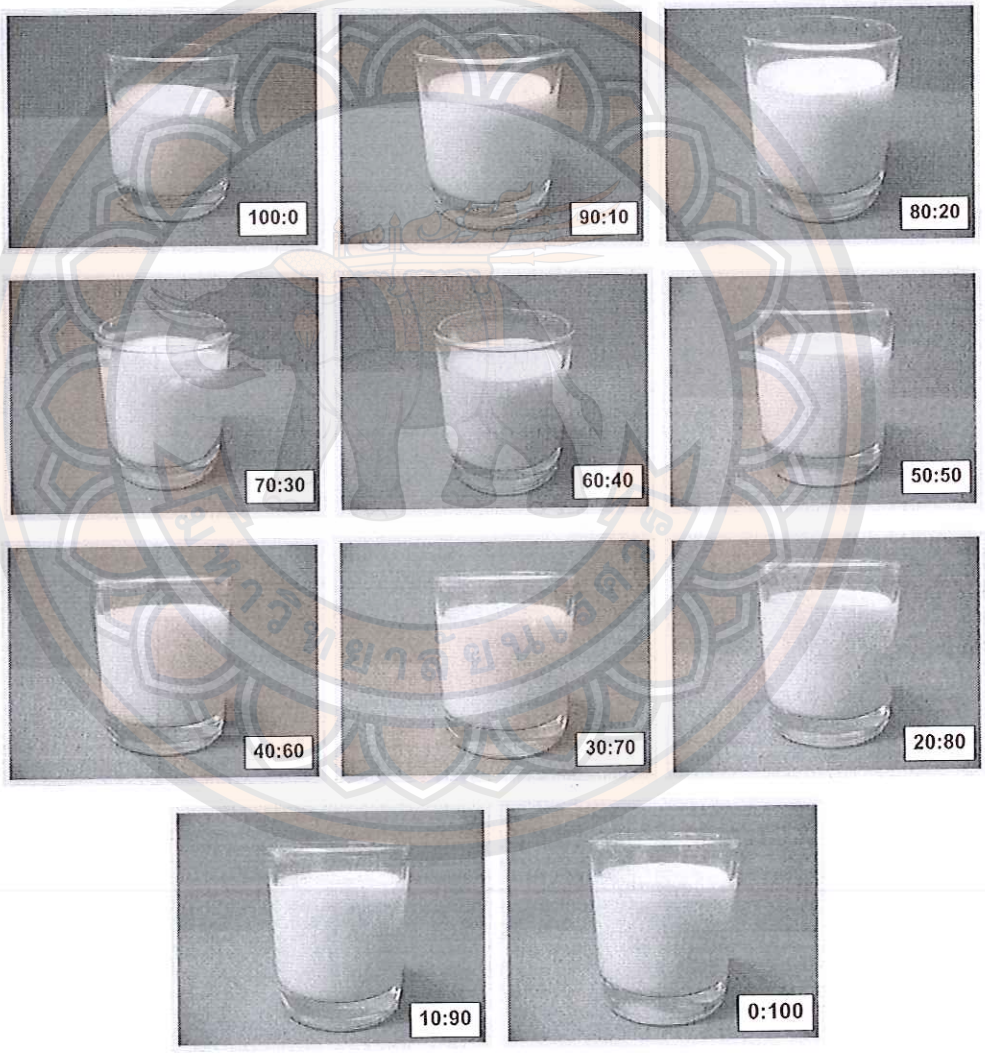

Figure 20 The appearance of 11 formula of LNDC

The appearance of mixed LNDC with coffee was shown in Figure 21. All 11 formulas of LNDC were brown color. The coffee had some scum on the coffee surface.

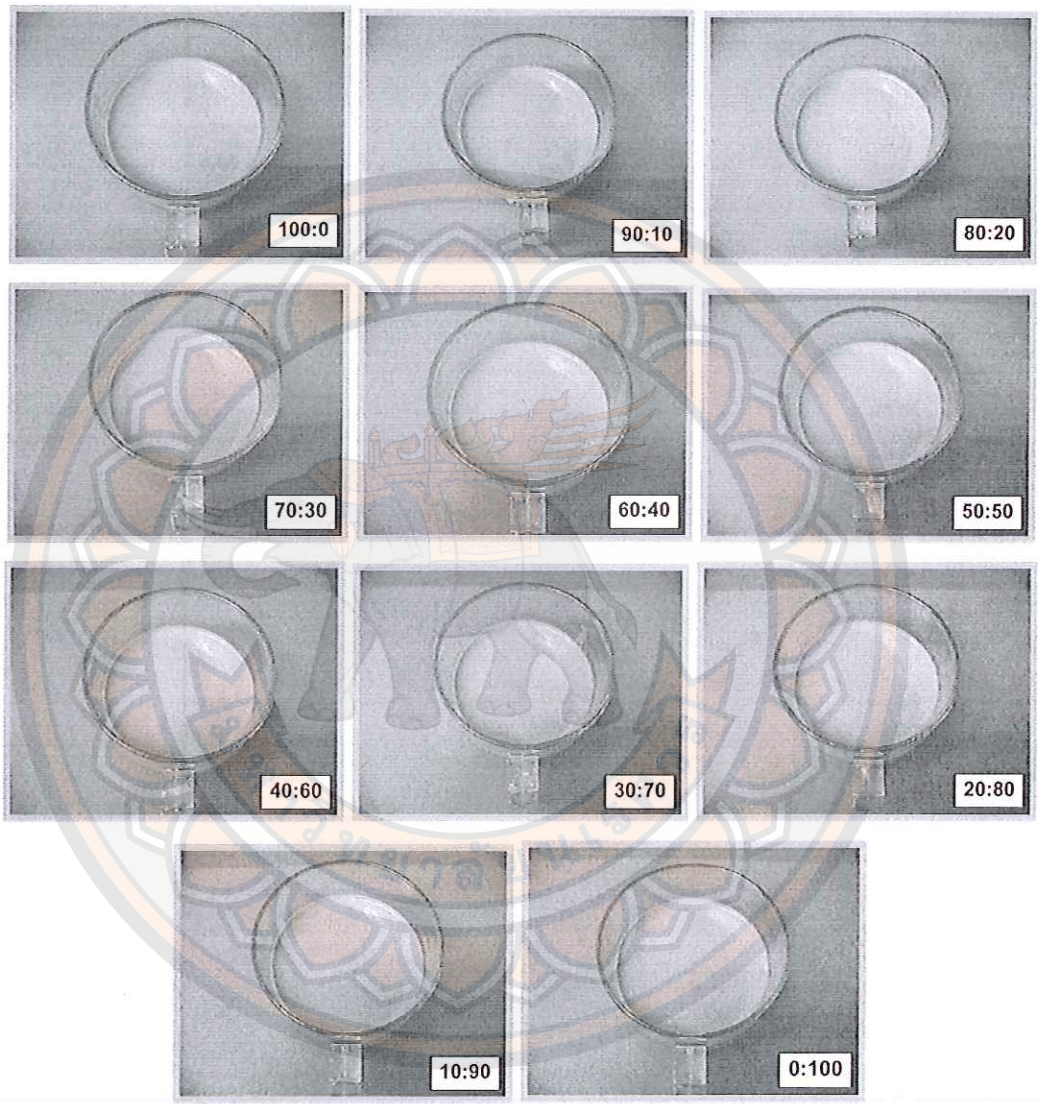

Figure 21 The appearance of coffee with 11 formula of LNDC added

Table 20 showed the color of LNDC which was white in color (L^* 93.06-86.25). Golde and Schmidt (2005) reported that the L^* for 9 formulations of coffee creamers were among 87.15-90.30. The LNDC of 100% CRBO was significantly more yellow (positive b^* value) than other mixed oil ($p \leq 0.05$). The LNDC had positive b^* values, indicating the yellow color. Negative values for a^* indicated that

the LNDC was more green than red (positive values for a* would indicate redness). Significant differences existed among the LNDC in a* values ($p \leq 0.05$). The viscosity of LNDC was 480 (100:0, 90:10, 60:40, 50:50, 10:90 and 0:100), 560 (80:20, 40:60 and 20:80) and 640 (70:30 and 30:70) cp. at 100 rpm, respectively. All the LNDC samples were more viscous than the mixed oil (Table 14) because LNDC have many ingredients that contributed viscosity, for example, glucose syrup and sodium caseinate which provided thickening properties.

Table 20 Color and viscosity of 11 formulas of LNDC

HPKO:CRBO	Color			Viscosity (cp.)
	L*	a*	b*	at 100 rpm
100:0	$93.06^{a}\pm0.01$	$1.34^{a}\pm0.05$	$4.26^{k}\pm0.02$	$480^{c}\pm9.32$
90:10	$92.12^{b}\pm0.03$	$0.80^{b}\pm0.02$	$6.47^{j}\pm0.04$	$480^{c}\pm9.30$
80:20	$91.22^{c}\pm0.12$	$0.23^{c}\pm0.01$	$9.14^{i}\pm0.01$	$560^{b}\pm7.20$
70:30	$90.37^{d}\pm0.02$	$0.01^{d}\pm0.01$	$10.87^{h}\pm0.02$	$640^{a}\pm5.25$
60:40	$89.63^{e}\pm0.13$	$-0.20^{e}\pm0.01$	$12.54^{g}\pm0.01$	$480^{b}\pm6.45$
50:50	$88.96^{f}\pm0.30$	$-0.45^{f}\pm0.02$	$14.10^{f}\pm0.02$	$480^{c}\pm9.30$
40:60	$88.48^{f}\pm0.30$	$-0.53^{g}\pm0.01$	$15.00^{e}\pm0.03$	$560^{b}\pm7.20$
30:70	$88.19^{f}\pm0.31$	$-0.66^{h}\pm0.03$	$15.96^{d}\pm0.01$	$640^{a}\pm5.50$
20:80	$87.66^{g}\pm0.12$	$-0.75^{i}\pm0.05$	$16.84^{c}\pm0.04$	$560^{b}\pm6.25$
10:90	$86.65^{h}\pm0.25$	$-0.84^{j}\pm0.01$	$18.29^{b}\pm0.02$	$480^{c}\pm10.20$
0:100	$86.25^{h}\pm0.30$	$-0.90^{k}\pm0.01$	$19.02^{a}\pm0.01$	$480^{c}\pm9.65$

Note: Means in the column with different superscripts were significantly different ($P \leq 0.05$)

1.2 Chemical properties of 11 formulas of LNDC

Acid value

The chemical properties of LNDC were shown in Figures 21-23. The acid value is a common parameter in the specification of fats and oils. It is defined as

the weight of KOH in mg needed to neutralize the organic acids present in 1 g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides. The acid value of 11 formulas of LNDC had value between 10-12 mg KOH/g samples (Figure 22). There is no standard on acid value for LNDC.

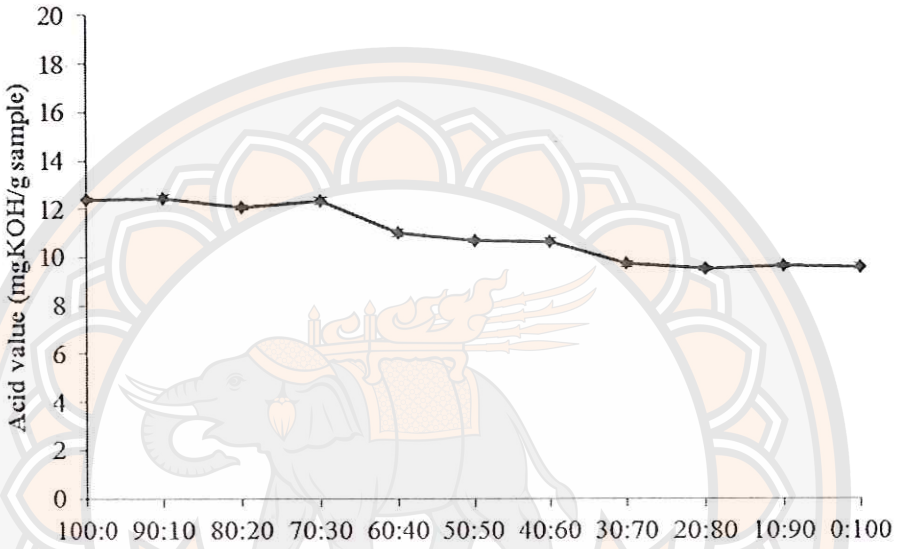

Figure 22 Acid value of 11 formulas of LNDC

Antioxidant Activity

Figure 23 showed the antioxidant activity of 11 formulas of LNDC. It was gradually decreased from 20.12 to 7.86%. This might be due to CRBO contained unsaturated fatty acids which could contribute to the increase of free fatty acids. The antioxidant properties of LNDC (Figure 23) were lower than the mixed oil (Table 19 and Figure 19c). This is because LNDC had more ingredients in the formula so that the antioxidant properties of LNDC were lower than mixed oil.

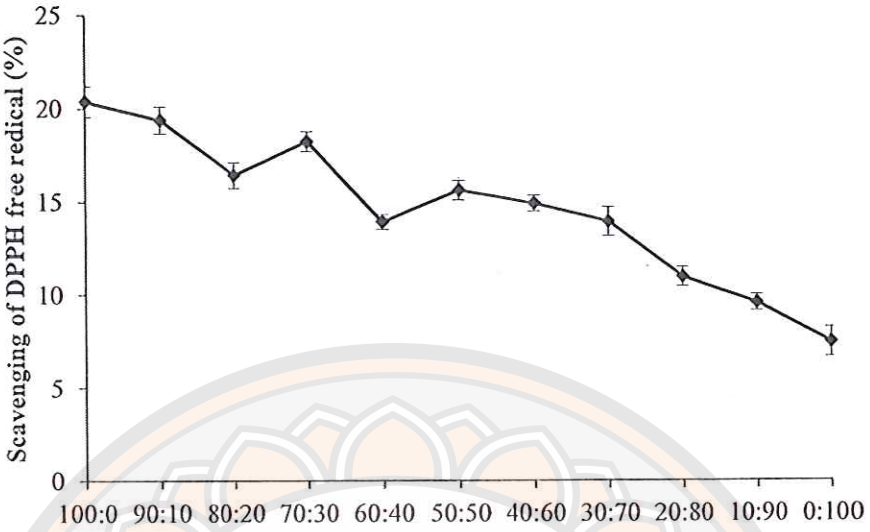

Figure 23 The antioxidant activity (DPPH) of 11 formulas of LNDC

Peroxide Value

Peroxide value of 11 formulas of LNDC was shown in Figure 24. Pure HPKO had the highest peroxide value ($p \leq 0.05$) whereas pure CRBO had the lowest peroxide value ($p \leq 0.05$). The peroxide value is a good indicator of the quality of fat and oil. Freshly refined fats should have hydroperoxide levels of less than 1 mEq/kg (Rosell, 1989). The limiting peroxide value specified by Joint FAO/WHO (1989) standards for refined oil is 10 mEq/kg. This study showed values of 0.78-1.7 mEq/kg for the pure CRBO and pure HPKO, respectively. There is no standard on peroxide value for LNDC.

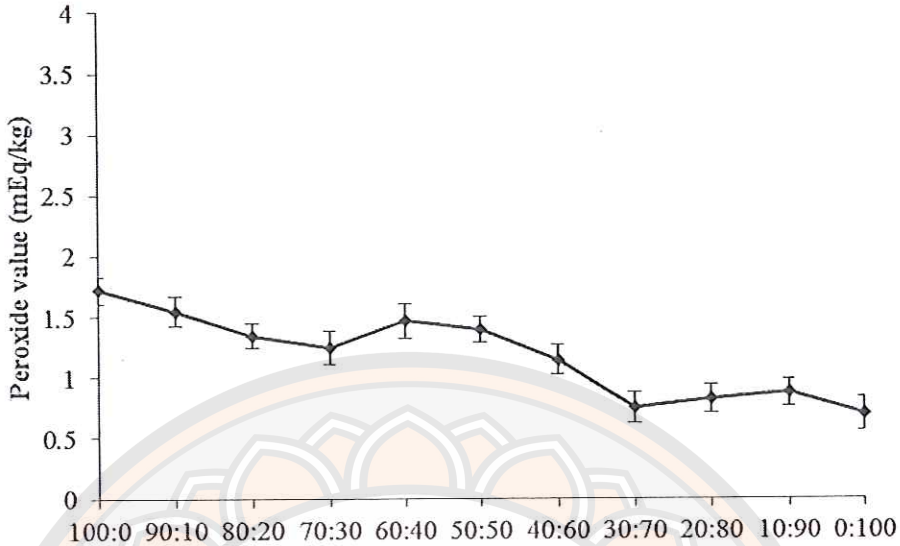

Figure 24 Peroxide value of 11 formulas of LNDC

1.3 Microbiological properties of 11 formulas of LNDC

The microbiological properties of LNDC were shown in Table 21. The total viable count, yeast and mold, coliform and *Escherichia coli* were not detectable in the LNDC. This indicated that the LNDC were safe for consumption.

Table 21 Microbiological counts of 11 formulas of LNDC

HPKO:CRBO	Total viable count (CFU/mL)^{ns}	Yeast and mold (CFU/mL)^{ns}	Coliform (MPN/mL)^{ns}	*Escherichia coli* (MPN/mL)^{ns}
100:0	<10	<10	<3	<3
90:10	<10	<10	<3	<3
80:20	<10	<10	<3	<3
70:30	<10	<10	<3	<3
60:40	<10	<10	<3	<3
50:50	<10	<10	<3	<3
40:60	<10	<10	<3	<3
30:70	<10	<10	<3	<3

Table 21 (cont.)

HPKO:CRBO	Total viable count (CFU/mL)^{ns}	Yeast and mold (CFU/mL)^{ns}	Coliform (MPN/mL)^{ns}	*Escherichia coli* (MPN/mL)^{ns}
20:80	<10	<10	<3	<3
10:90	<10	<10	<3	<3
0:100	<10	<10	<3	<3

Note: ^{ns} Means in the column were not significantly different (P>0.05)

1.4 Sensory evaluation of 11 formulas of LNDC

All 11 formulas of LNDC were sensory tested by 50 untrained panelist (whose like and drink coffee) using the 9-points Hedonic scale (Score: 1 = dislike extremely, 9 = like extremely) consisting of students and staffs of the Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand. Attributes of LNDC tested were color of cream, liquid suspension, odor of cream, color of cream, taste of coffee, texture of coffee and overall liking. Each panelist was served at 10 am with 15 mL of LNDC in a plastic cup, 2 g of coffee, 8 g of sugar and 70 g of hot water. The results of sensory evaluation test were shown in Figure 25. In each attribute, the 3 highest scores were selected to develop in the next step by using a mixture design. The 3 highest scores in color of LNDC were 50:50, 30:70 and 20:80. The 4 highest scores in liquid suspension were 60:40, 50:50, 10:90 and 0:100. The 3 highest scores in odor of liquid were 40:60, 30:70 and 20:80. The 4 highest scores in color of coffee were 60:40, 50:50, 10:90 and 0:100. The 3 highest scores in taste of coffee were 60:40, 50:50 and 20:80. The 3 highest scores in texture of coffee were 60:40, 50:50 and 20:80. The 3 highest scores in overall liking were 60:40, 50:50 and 20:80. From these results, it could be concluded that LNDC of 20:80 mixed oil had higher sensory scores than other LNDC formulas. Therefore, it was selected for further formula development by using a mixture design.

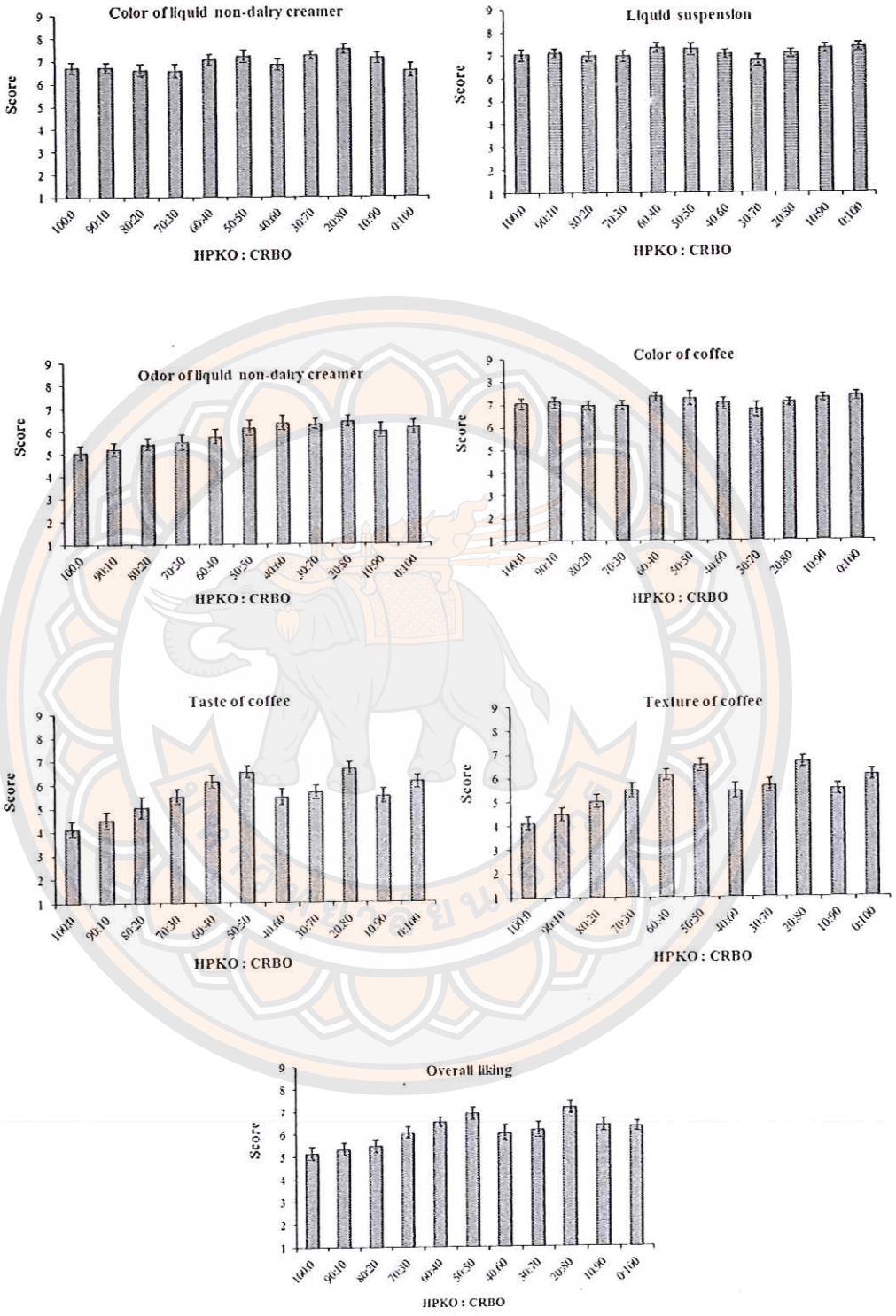

Figure 25 The liking scores of 11 formulas of LNDC

Development of LNDC by using a Mixture Design

In this part, the selected ratio of mixed oil for the production of LNDC of 20:80 was used. Mixture design for development of LNDC was necessary since the surface of coffee still had scum, indicating that formula adjustment was needed. Mixture design presented 7 formulas by variation of glucose syrup, mixed oil and sodium caseinate as shown in Table 22.

Table 22 Mixture design for development of LNDC

Formula	Glucose syrup (g) (30-50%)*	Mixed oil (g) (30-40%)*	Sodium caseinate (g) (1-10%)*
1	129.5	104	27
2	78	156.6	27
3	129.5	131	0
4	103.65	156.6	0
5	110	137	13.6
6	78	182.7	0
7	103.65	143.55	13.6

Note: *The range of ingredient which added in LNDC

Figures 26 and 27 showed the appearance of 7 formulas of LNDC after adjusting the main ingredients and mixing LNDC with coffee. The appearance of 7 formulas of LNDC did not show differences in color but after mixed LNDC with coffee, formulas 1, 2, 5 and 7 had a little amount of scum. On the other hand, formulas 3, 4 and 6 had a lot of scum. All 7 formulas of LNDC could reduce the color of coffee to be more soften and lighten.

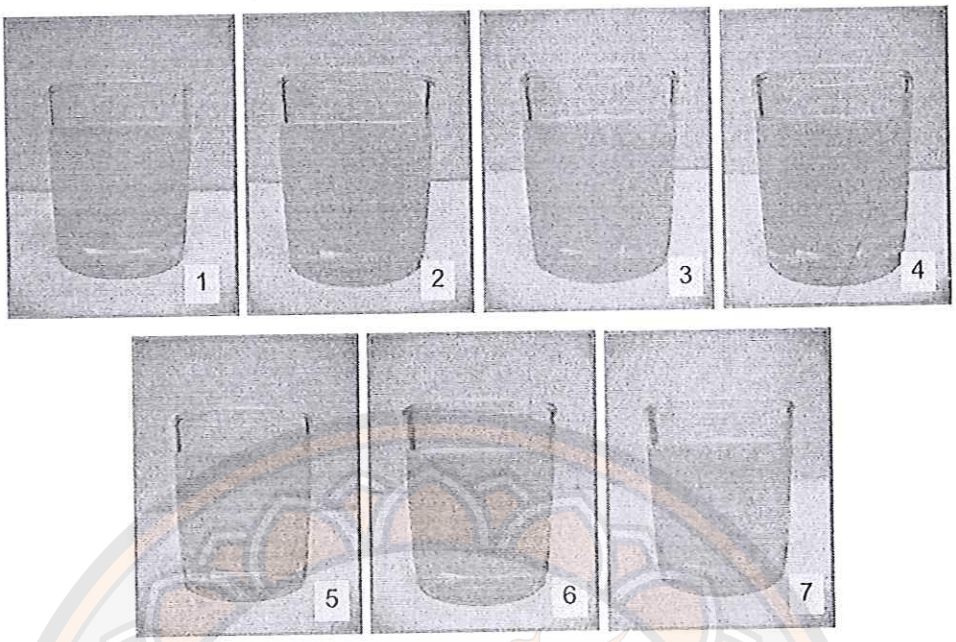

Figure 26 The appearance of 7 formulas of LNDC after adjustment from mixture design

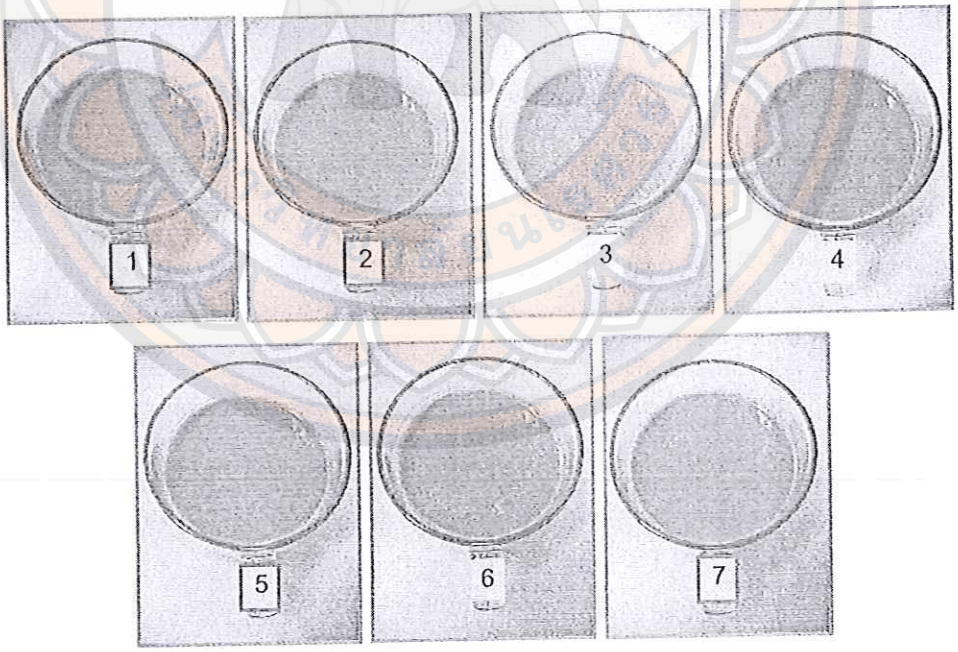

Figure 27 The appearance of 7 formulas of LNDC mixed with coffee after adjustment from mixture design

All 7 formulas of LNDC were sensory tested by 50 untrained panelists (whose like and drink coffee) using the 9-points Hedonic scale (Score: 1 = dislike extremely, 9 = like extremely) consisting of students and staffs of the Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand. Attributes of LNDC were color of cream, liquid suspension, odor of cream, color of cream, taste of coffee, texture of coffee and overall liking. Each panelist was served at 10 am with 15 ml of LNDC in a plastic cup, 2 g of coffee, 8 g of sugar and 70 g of hot water. The formula with highest score was selected as the best formula of LNDC. The liking scores of all attributes were shown in Figure 28. The formula 7 had the highest score in all attributes so that it was regarded as the best formula of LNDC.

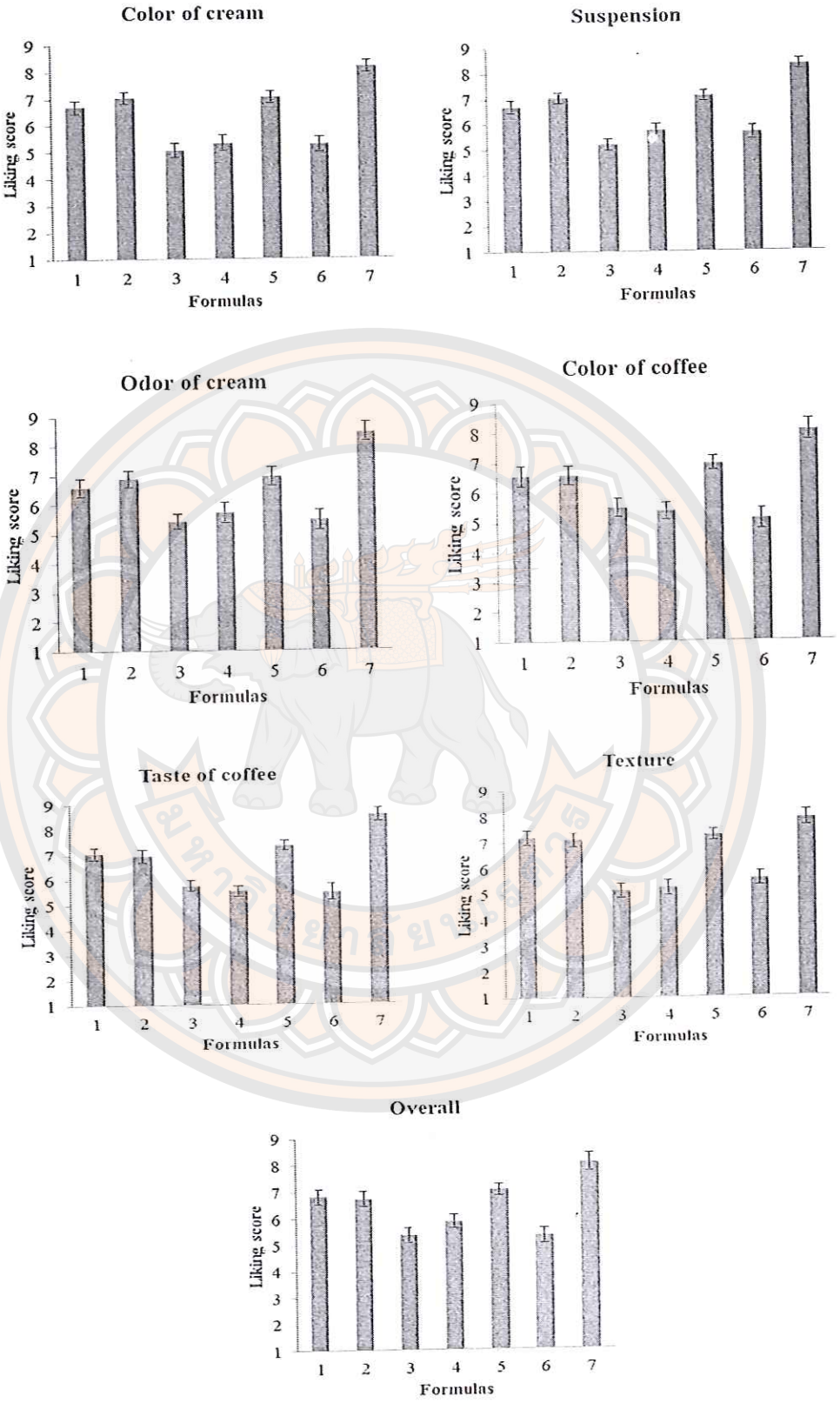

Figure 28 The liking scores of 7 formulas of LNDC after adjustment from a mixture design

2. Process and properties of 11 formulas of PNDC

2.1 Physical properties of 11 formulas of PNDC

The characteristics of PNDC were presented in Figure 29. All of formula had bigger size of granules than the control. PNDC of all treatments had yellowish color and moist powder.

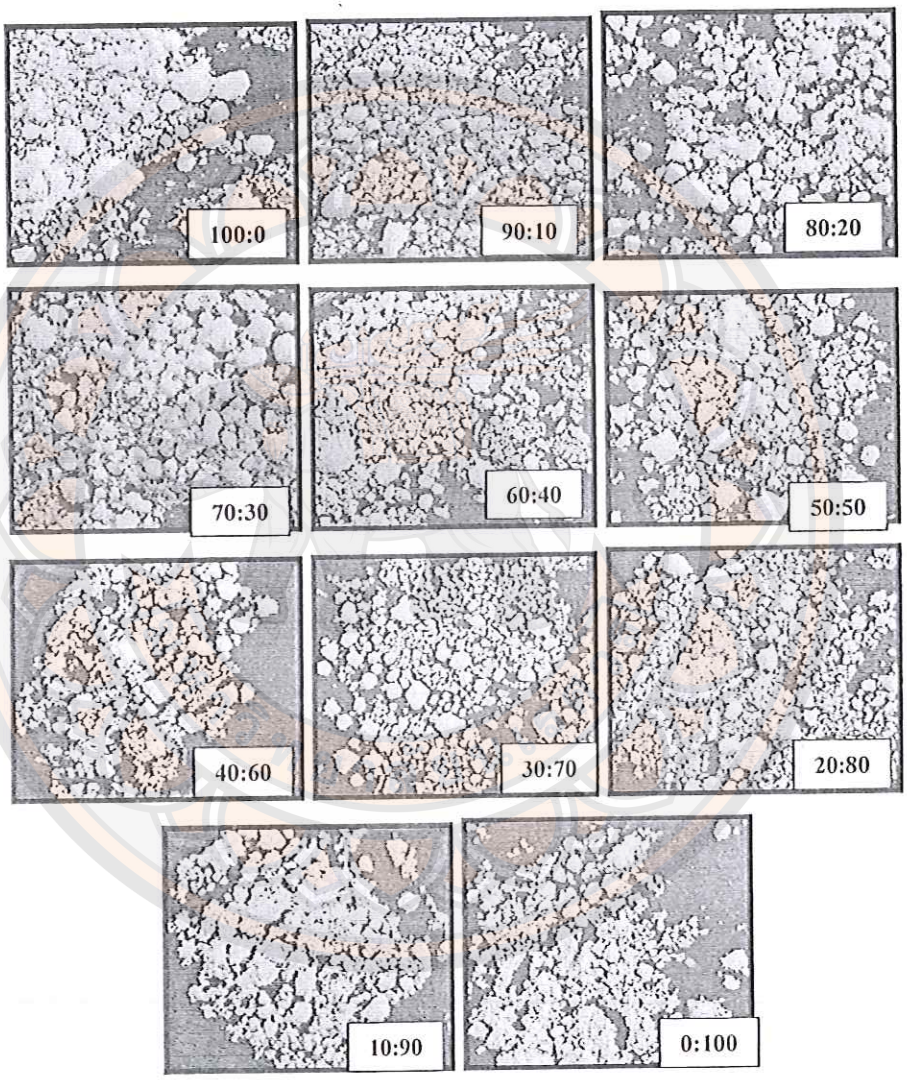

Figure 29 The appearance of PNDC after taking out of the spray dryer

Tables 23 showed the yield of PNDC obtained from using 2 spray driers. The experiment was conducted at 2 places. Figure 13 was the spray dryer in laboratory of Department of Agro-Industry, Naresuan University for development of formula of PNDC and the yield was shown in Table 23. Another spray drier was from Perfect Natural Food Powder and Flavor 2002 (Thailand) (PNF) Company, Samusakorn, Thailand, and the yield of using this spray dryer was shown in Table 23.

Table 23 The yield of PNDC after spray dried process from lab scale and PNF Company

Formula	Lab scale (Yield %)	PNF Company (Yield %)
0:100	34.48	49.36
10:90	41.78	85.00
20:80	34.50	61.01
30:70	37.38	66.67
40:60	37.61	2.21
50:50	30.89	4.35
60:40	36.31	19.16
70:30	41.94	16.27
80:20	35.34	10.84
90:10	34.66	47.19
100:0	35.27	48.72

The bulk density, color and solubility of PNDC were shown in Table 24. Bulk density of all treatments was not significant different ($p>0.05$) with the value of 0.32-0.39 g/cm^3. Color of all PNDC was light yellow. The solubility of PNDC for all treatment was not significant difference ($p>0.05$) and was between 71-75 seconds.

Table 24 Bulk density, color and solubility of 11 formulas of PNDC

HFKO: CRBO	Bulk density (g/cm^3)^{ns}	Color			Solubility (second)^{ns}
		L*	a*	b*	
100:0	0.35±0.03	80.01^{d}±0.01	-0.98^{a}±0.05	11.14^{k}±0.02	72.00±2.50
90:10	0.37±0.06	80.82^{c}±0.03	-0.88^{b}±0.02	10.72^{j}±0.04	73.18±2.70
80:20	0.38±0.04	79.97^{d}±0.12	-1.06^{c}±0.01	8.39^{i}±0.01	75.34±3.10
70:30	0.34±0.02	81.04^{b}±0.02	-0.94^{d}±0.01	8.95^{h}±0.02	71.46±3.00
60:40	0.37±0.05	79.30^{e}±0.13	-1.05^{e}±0.01	8.97^{g}±0.01	72.56±2.20
50:50	0.39±0.03	81.61^{a}±0.30	-0.97^{f}±0.02	7.43^{f}±0.02	70.98±3.20
40:60	0.39±0.01	76.73^{f}±0.30	-1.05^{g}±0.01	9.45^{e}±0.03	73.59±3.10
30:70	0.32±0.04	76.76^{f}±0.31	-0.94^{h}±0.03	8.47^{d}±0.01	74.61±2.40
20:80	0.34±0.05	81.48^{a}±0.12	-1.01^{i}±0.05	7.92^{c}±0.04	74.63±2.80
10:90	0.34±0.02	81.52^{a}±0.25	-1.13^{j}±0.01	7.35^{b}±0.02	73.64±2.30
0:100	0.38±0.06	81.43^{a}±0.30	-1.08^{k}±0.01	7.85^{a}±0.01	75.15±3.10

Note: Means in the column with different superscripts were significantly different ($P \leq 0.05$)

Figure 30 presented characteristics of PNDC by using Stereo Compound Microscope. All of 11 formulas of PNDC had larger particle size. The particle size of PNDC was agglomerated. The scum of PNDC was occurred on surface of coffee (Figure 31) when added it into liquid coffee. The scum was presented all coffee surface of mixed oil ratio 60:40-0:100. The scum of mixed oil ratio 100:0-70:30 was shown on some area of the coffee surface. Amount of scum was shown in Figure 32. The scum of PNDC with mixed oil ratio 100:0-70:30 had higher level than other ratios.

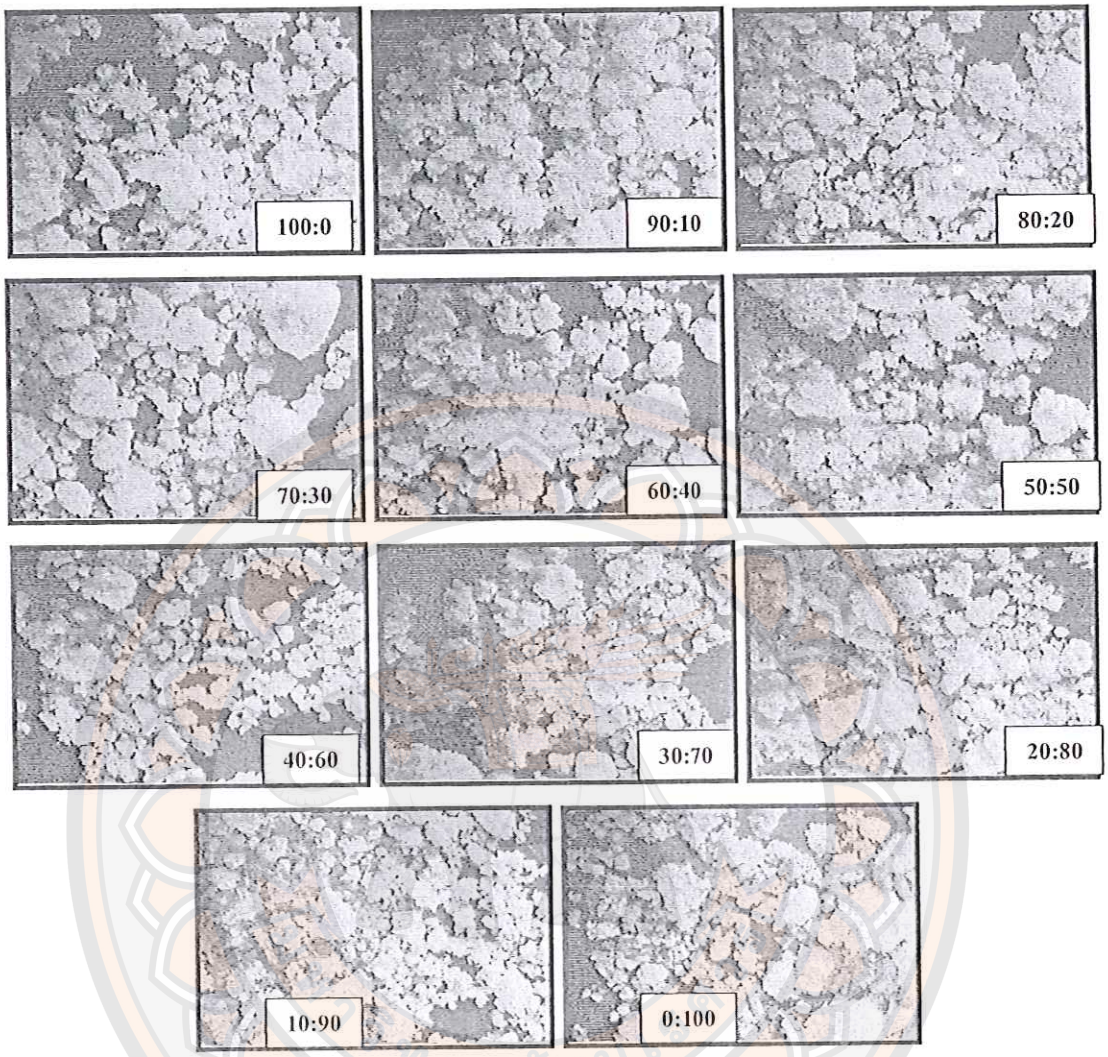

Figure 30 Characteristic of PNDC by using Stereo Compound Microscope (4X)

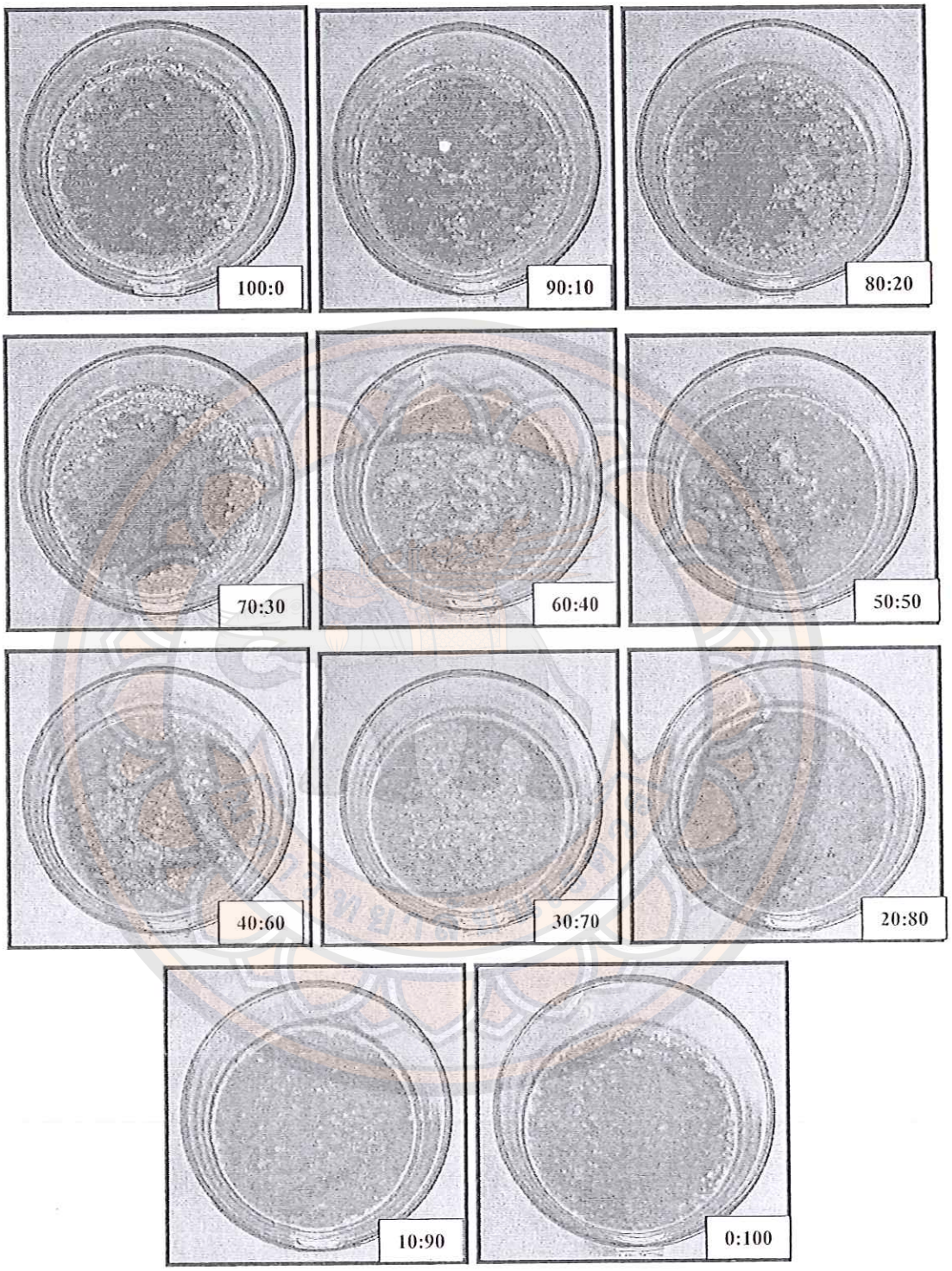

Figure 31 The appearance of 11 formulas of PNDC after mixing with coffee

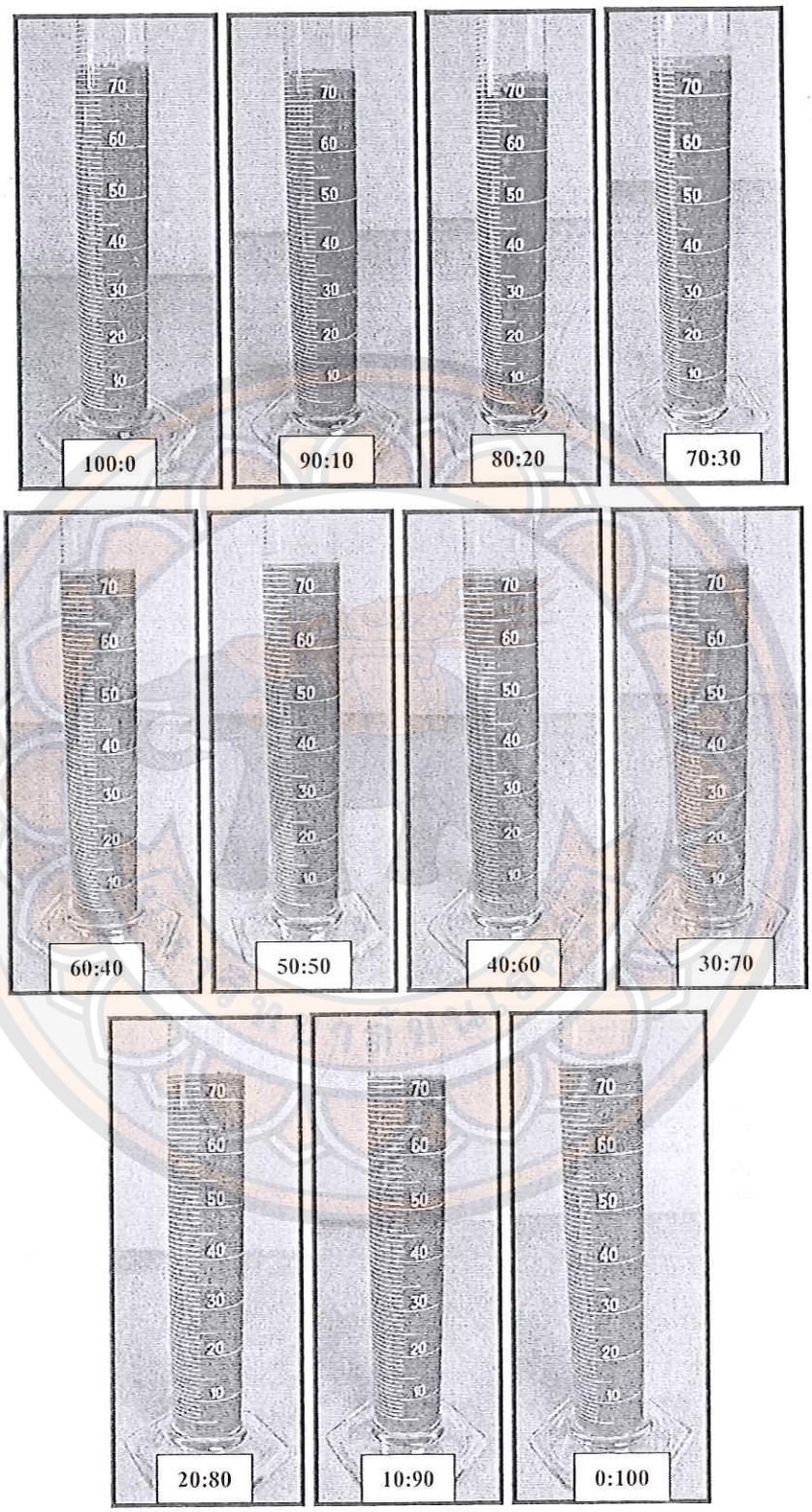

Figure 32 The volume of scum occurred in 11 formulas of PNDC after mixing with coffee

2.2 Chemical properties of 11 formulas of PNDC

The chemical properties of PNDC were shown in Figures 33-38. All of chemical properties were slightly decrease when the ratio of CRBO increase.

Moisture Content

Figure 33 showed the moisture content of the spray-dried powder. The mixing ratio of oil showed a significant effect on moisture content. Moisture content represents the water composition in a food system. The moisture content of spray-dried powder decreased with the increase in CRBO. Because the CRBO contained liquid phase less than HPKO. It was similar to the results of water activity.

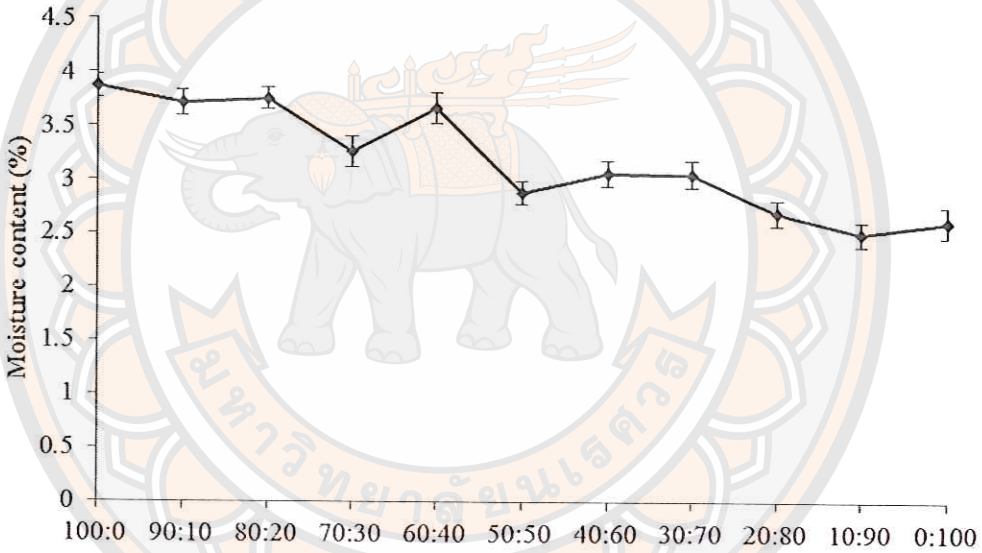

Figure 33 Moisture content of 11 formulas of PNDC

Water Activity (a_w)

Water activity measures the activity of free water in a food system which is responsible for any biochemical reactions. Higher water activity indicates that there is more free water available for biochemical reactions. Basically, food with a_w less than 0.6 is microbiologically stable. From Figure 34, the water activity of the powder produced was in the range of 0.17-0.31. This showed that all the treatments were microbiologically stable. The water activity of PNDC significantly decreased ($p \leq 0.05$) with the increase of CRBO. This result was in agreement with the results of the moisture content of PNDC.

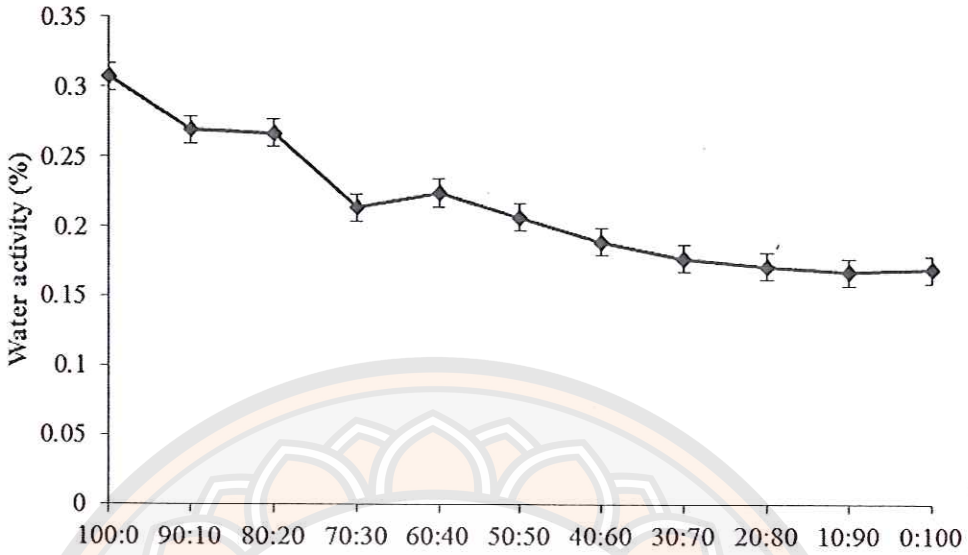

Figure 34 Water activity of 11 formulas of PNDC

Antioxidant Activity

DPPH is a free radical compound that has been popularly used to determine the free radical scavenging activity of samples (Babbar, et al., 2011). The DPPH radical scavenging increased with increasing ratio of CRBO was shown in Figure 35. It was found that percentage of DPPH radical scavenging of CRBO was highest (25%) ($p \leq 0.05$). In contrast, the percentage of DPPH radical scavenging of HPKO was lowest (5%) ($p \leq 0.05$). This result was in agreement with the antioxidant activity of mixed oil shown in Table 19.

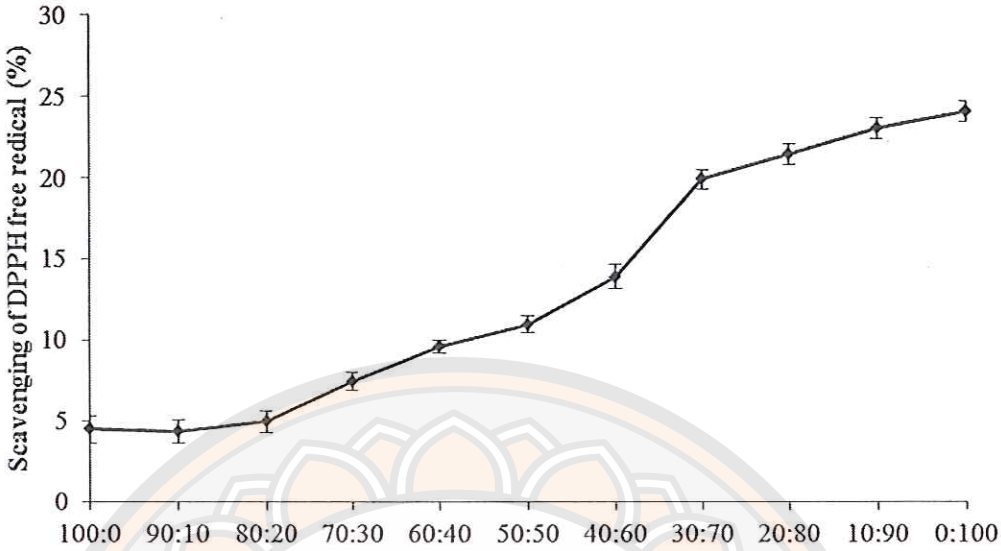

Figure 35 The antioxidant activity (DPPH) of 11 formulas of PNDC

Acid Value

The acid value was decreased with increasing ratio of CRBO. The acid values of 11 formulas of PNDC were between 10-12 mg KOH/g samples (Figure 36). Hydrolytic rancidity was one of the causes of rancidity with water being catalyst. From Figures 32 and 33, since the moisture content and water activity of HPKO were higher than that of CRBO, HPKO had higher acid value than CRBO. There is no standard on acid value for PNDC.

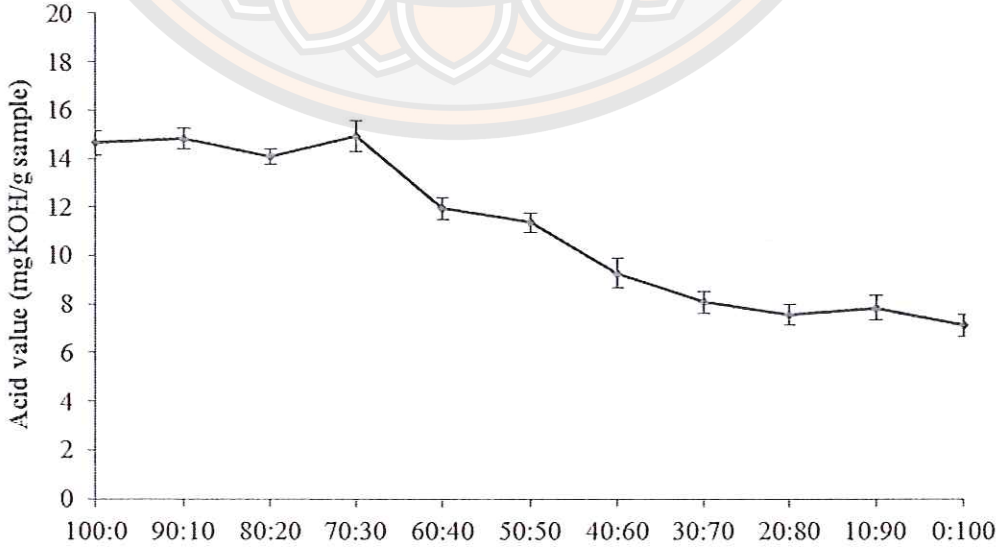

Figure 36 Acid value of 11 formulas of PNDC

Peroxide Value

Peroxide value of 11 formulas was shown in Figure 37. The peroxide value was decreased when increased the ratio of CRBO. It was indicated that CRBO contained some antioxidants such as γ-oryzanol and vitamin E that could prevent peroxide production. The peroxide value is a good indicator of the quality of fat and oil. Freshly refined fats should have hydroperoxide levels of less than 1 mEq/kg (Rosell, 1989). The limiting peroxide value specified by Joint FAO/WHO (1989) standards for refined oil is 10 mEq/kg. This study showed values of 2.85-0.48 mEq/kg for the pure HPKO and pure CRBO, respectively. There is no standard on peroxide value for PNDC.

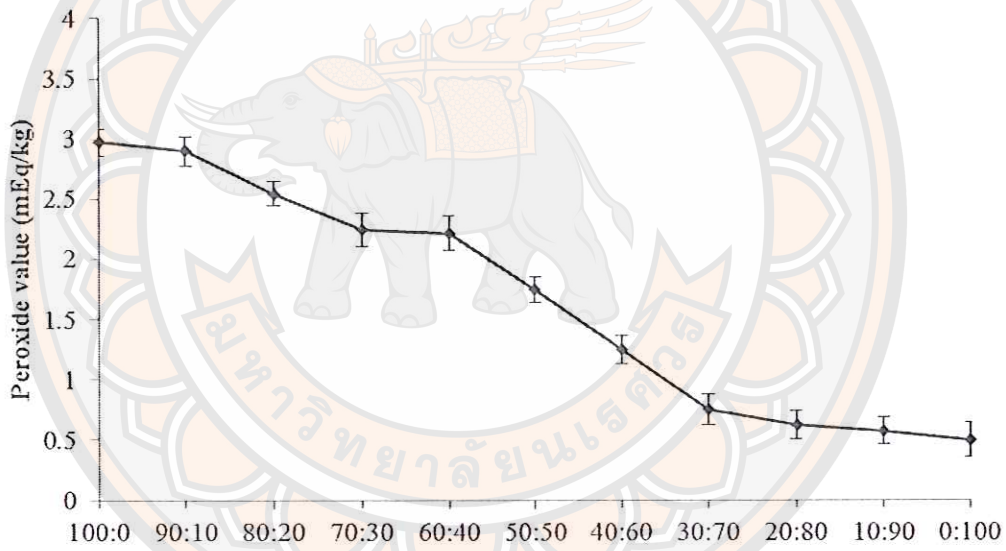

Figure 37 Peroxide value of 11 formulas of PNDC

Acidity (as lactic acid)

Acidity of 11 formulas was shown in Figure 38. The acidity was decreased when increased the ratio of CRBO. Because HPKO was containing water that cause to hydrolytic rancidity and CRBO had high antioxidant substrate to preventing rancidity.

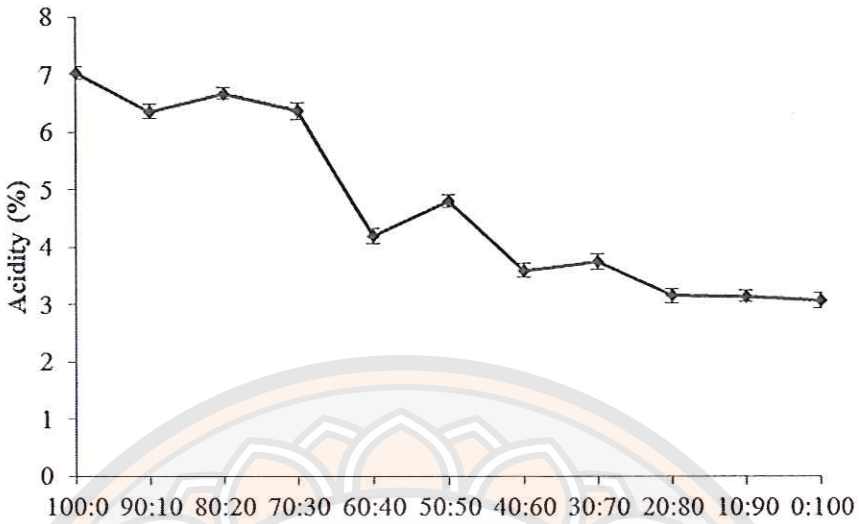

Figure 38 Acidity (as lactic acid) of 11 formulas of PNDC

2.3 Microbiological properties of 11 formulas of PNDC

The microbiological properties of PNDC were shown in Table 25. The total viable count, yeast and mold, coliform and *Escherichia coli* were not detectable in PNDC. This result indicated that the PNDC was safe for consumption.

Table 25 Microbiological counts of 11 formulas of PNDC

HPKO:CRBO	Total viable count (CFU/mL)^{ns}	Yeast and mold (CFU/mL)^{ns}	Coliform (MPN/mL)^{ns}	*Escherichia coli* (MPN/mL)^{ns}
100:0	<10	<10	<3	<3
90:10	<10	<10	<3	<3
80:20	<10	<10	<3	<3
70:30	<10	<10	<3	<3
60:40	<10	<10	<3	<3
50:50	<10	<10	<3	<3
40:60	<10	<10	<3	<3
30:70	<10	<10	<3	<3
20:80	<10	<10	<3	<3

Table 25 (cont.)

HPKO:CRBO	Total viable count (CFU/mL)^{ns}	Yeast and mold (CFU/mL)^{ns}	Coliform (MPN/mL)^{ns}	*Escherichia coli* (MPN/mL)^{ns}
10:90	<10	<10	<3	<3
0:100	<10	<10	<3	<3

Note: ^{ns} Means in the column were not significantly different (P>0.05)

2.4 Sensory evaluation of 11 formulas of PNDC

All 11 formulas of PNDC were sensory tested by 50 untrained panelists (whose like and drink coffee) using the 9-points hedonic scale (Score: 1 = dislike extremely, 9 = like extremely) consisting of student and staffs of the Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand. Attributes of PNDC were color of PNDC, powder suspension, odor of PNDC, color of coffee, taste of coffee, texture of coffee, dispersibility and overall liking. Each panelist was served at 10 am with 3 g of PNDC in a plastic cup, 2 g of coffee, 8 g of sugar and 70 g of hot water. The results of sensory evaluation test were shown in Figure 38. In each attribute, the 3 highest scores were selected for further development by using a mixture design. The 3 highest scores in color of powder were 100: 0, 90:10 and 10:90. The 3 highest scores in odor of powder were 100: 0, 90:10 and 80:20. The 3 highest scores in dispersibility were 40:60, 20:80 and 10:90. The 5 highest scores in color of coffee were 80:20, 70:30, 20:80, 10:90 and 0:100. The 3 highest scores in powder of suspension were 70:30, 50:50 and 0:100. The 6 highest scores in taste of coffee were 100;0, 90:10, 60:40, 50:50, 10:90 and 0:100. The 3 highest scores in texture of coffee were 90:10, 50:50 and 0:100. The 4 highest scores in overall liking were 80:20, 50:50, 20:80 and 10:90. From these results, it could be concluded that PNDC of 10:90 mixed oil had higher sensory scores than other PNDC formulas. Therefore, it was selected for further formula development by using a mixture design.

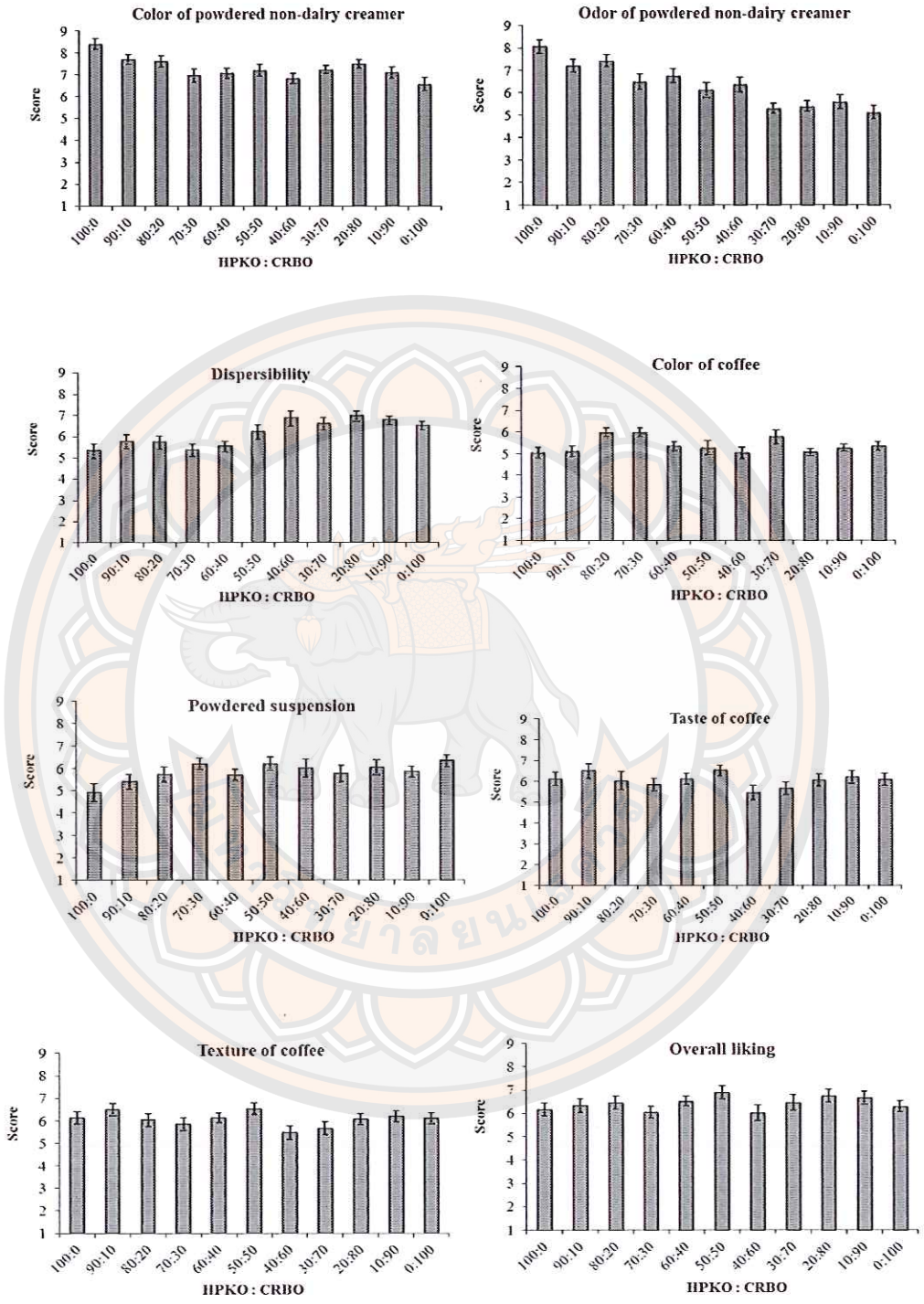

Figure 39 The liking scores of 11 formulas of PNDC

Development of PNDC by using a mixture design

In this part, the selected mixed oil for PNDC was containing 10% HPKO and 90% CRBO was used. Mixed oil was brown color and liquid state at room temperature. PNDC was white color and had rice bran odor. Mixture design for development of PNDC was necessary because the surface of coffee still had scum, indicating that formula adjustment was needed. Mixture design presented 5 formulas by varying glucose syrup, mixed oil, skim milk and lecithin (Table 26).

Table 26 Mixture design for development of PNDC

Formula	Glucose syrup (g) (30-50%)*	Rice bran oil (g) (30-40%)*	Skim milk (g) (1-10%)*	Lecithin (g) (5-8%)*
1	152	80	35	25.25
2	114.5	118	59.5	0
3	96	170.65	6.5	20
4	152	118	22.45	0
5	96	170.65	0	25.25

Note: *The range of ingredient which added in PNDC

Figure 40 showed the PNDC of 5 formulas after mixing with coffee. Formulas 2 and 4 had the scum on the coffee surface. Formulas 3 and 5 had the oil droplets on the coffee surface. Therefore, formulas 2, 3, 4 and 5 were not regarded as the suitable formula.

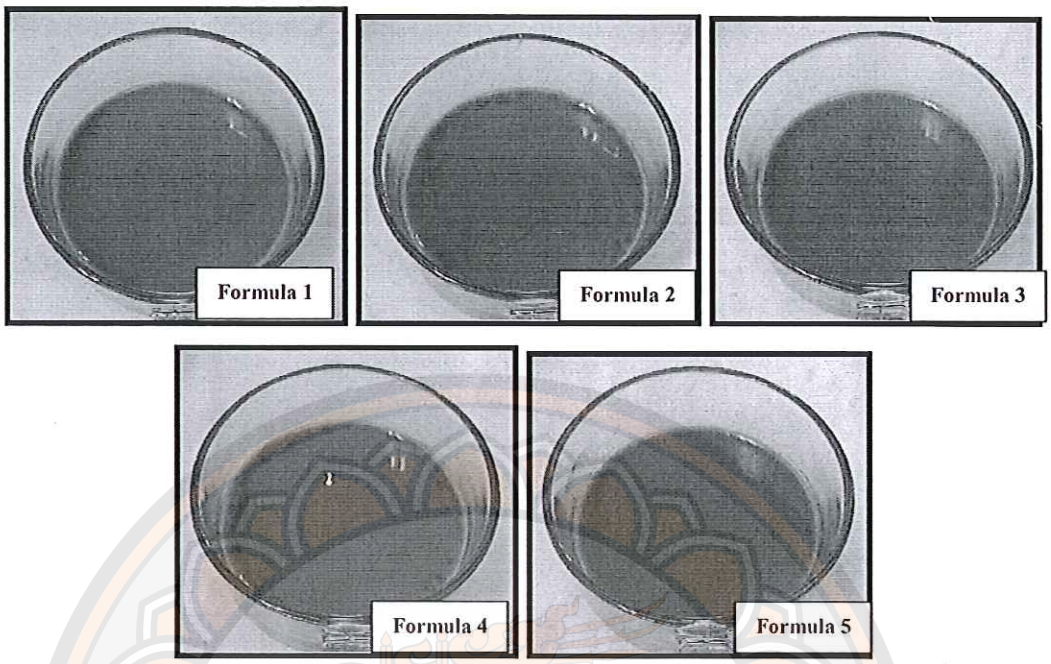

Figure 40 The appearance of 5 formulas of PNDC mixed with coffee after adjustment from mixture design

All 5 formulas of PNDC were sensory tested by 50 untrained panelists (whose like and drink coffee) using the 9-points hedonic scale (Score: 1 = dislike extremely, 9 = like extremely) consisting of student and staffs of the Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Thailand. Attributes of PNDC were color of powder, powder suspension, dispersibility, odor of powder, color of coffee, taste of coffee, texture of coffee and overall liking. Each panelist was served at 10 am with 3 g of PNDC in a plastic cup, 2 g of coffee, 8 g of sugar and 70 g of hot water. The highest score was the best formula of PNDC. The liking scores of all attributes were shown in Figure 40. The formula 1 showed the highest score in all attributes except odor of powder, so that it was regarded as the best formula of PNDC.

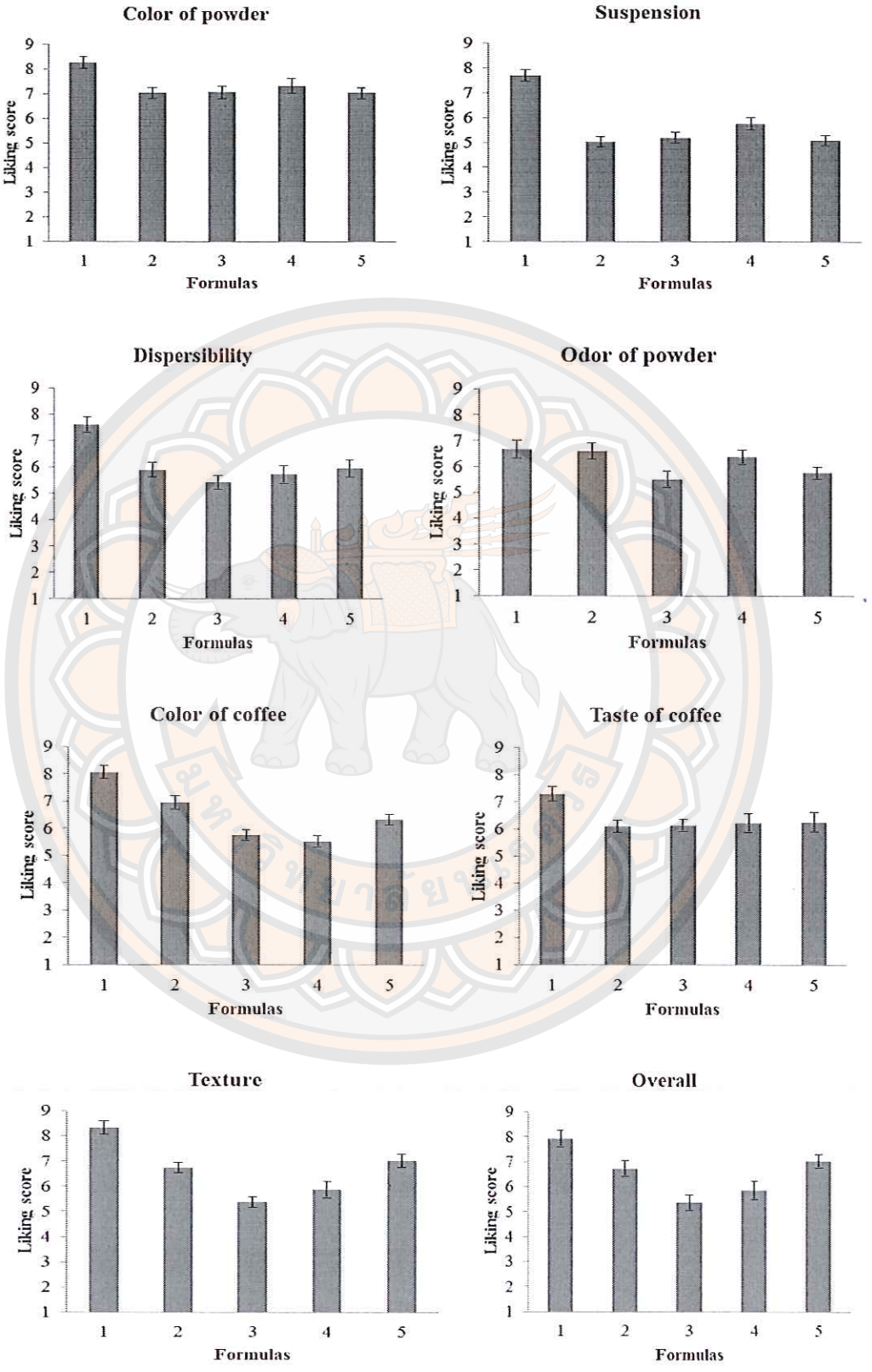

Figure 41 The liking score of 5 formulas of PNDC after adjustment from mixture design

3. Cost analysis

For cost analysis, it was aimed to find out whether it is financially possibly to manufacture LNDC and PNDC from mixed HPKO and CRBO.

3.1 Cost analysis of LNDC

From the developed formula of LNDC the price of raw material per unit can be calculated as following (Table 27).

Table 27 Cost of ingredients for liquid non-diary creamer

Ingredients	Price/unit	Price/formula (Baht)
Water	15 Baht/20 L	0.15
Rice bran oil	500 Baht/kg	92.80
Palm kernel oil	56 Baht/kg	2.24
Glucose syrup	60 Baht/kg	7.20
Sodium caseinate	980 Baht/kg	23.52
Maltodextrin	190 Baht/kg	1.52
Potassium phosphate	28 Baht/kg	0.0168
	Total	127.44

1 formula yielded LNDC 430 ml and costed 127.44 Baht

So 1 ml = 127.44/430

= 0.296 Baht

1 serving size (15 ml) = 15×0.296 = 4.44 Baht

Therefore, 1 serving size (15 mL) of LNDC costed 4.44 Baht.

3.2 Cost analysis of PNDC

From the selected formula of PNDC, the price of raw material per unit was calculated as following (Table 28).

Table 28 Cost of ingredients for powdered non-diary creamer

Ingredients	Price/unit	Price/formula (Baht)
Glucose syrup	60 Baht/kg	33.60
Rice bran oil	500 Baht/kg	187.92
Palm kernel oil	56 Baht/kg	2.01
Skim milk powder	299 Baht/700g	52.50
Lecithin	500 Baht/kg	54.87
Maltodextrin	190 Baht/kg	4.75
Sodium caseinate	980 Baht/kg	24.50
Water	15 Baht/20 L	0.75
	Total	360.90

1 formula yielded PNDC 350.22 g and costed 360.90 Baht

So 1 g = 350.22/360.90

= 0.97 Baht

1 serving size (3 g) = 0.97 ×3 = 2.91 Baht

Therefore, 1 serving size (3 g) of PNDC costed 2.91 Baht.

Part 3 The shelf life of liquid and powdered non-dairy creamer from mixture of hydrogenated palm kernel oil and cold pressed rice bran oil

This part aimed to find out the quality stability of the selected LNDC and PNDC from mixed HPKO and CRBO during storage.

The selected LNDC and PNDC from mixed HPKO and CRBO were packed in a aluminium foil bag at 3 g as the commercial ones and kept at refrigerated (4±2°C) for 14 days for LNDC and accelerated shelf life at 30 and 40°C for 80 days for PNDC. The LNDC samples were randomly examined every 3 days whereas the PNDC samples were randomly tested every 10 days. The properties tested were as following:

1. Quality properties of LNDC during storage time

The quality of LNDC was determinated in physical, chemical, microbiological and sensory evaluation properties. The finished product of LNDC was yellow color.

1.1 Physical properties of LNDC during storage time

Color (L*, a*, b* and ΔE)

The lightness (L*), a*, b* and ΔE of LNDC did not change during storage at 4±2°C for 12 days (Figure 42), indicating that storage temperature of 4±2°C had no effect on the color of LNDC. Golde and Schmidt (2005) reported that L* of 9 formulations of coffee creamers were 87.15-90.30 which were similar to the result of this study.

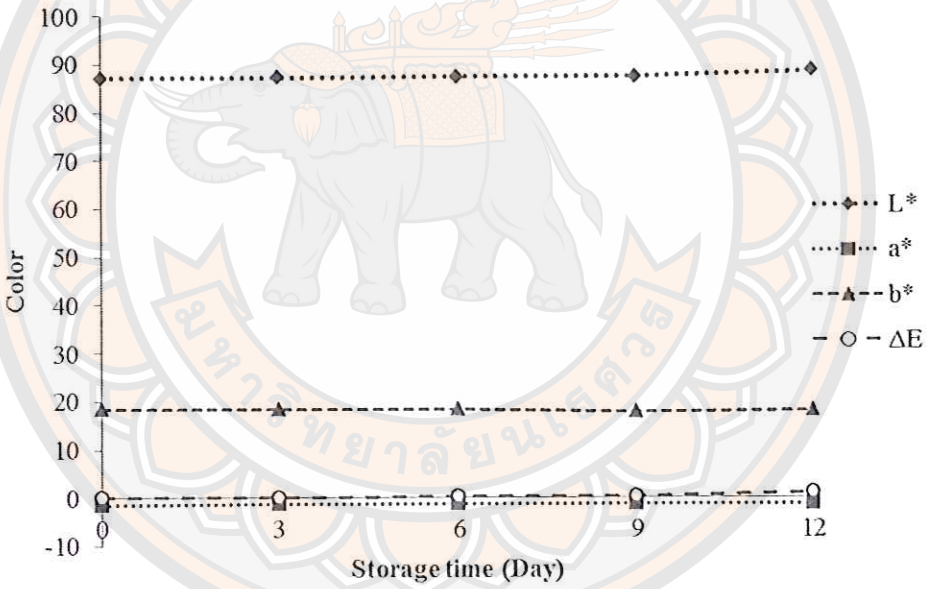

Figure 42 Color (L*, a*, b* and ΔE) of liquid non-dairy creamer during storage at 4±2°C for 12 days

Viscosity

Figure 43 shown viscosity of liquid non-dairy creamer during storage at 4±2°C for 12 days. The viscosity of LNDC was increased during storage for 12 days (820 to 1,120 cp).

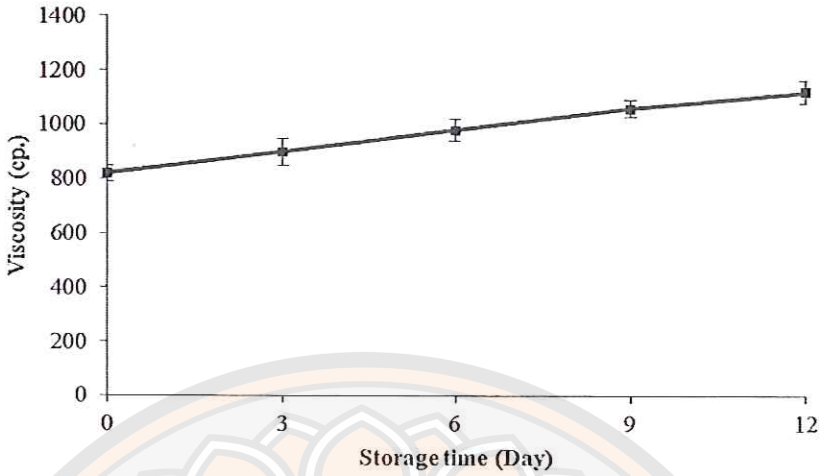

Figure 43 Viscosity of liquid non-dairy creamer during storage at 4±2°C for 12 days

1.2 Chemical properties of LNDC during storage time

The change of chemical properties of LNDC during storage at 4±2°C for 12 days was shown in Figures 44, 45 and Table 29. Antioxidant activity, moisture and fat content were slightly changed but not significantly difference ($p>0.05$) with time of storage (Figure 44). Acidity and peroxide value were slightly increased from day 0 to day 12 (1.936-4.353% and 1.96-4.92 mEq/kg, respectively) (Figure 45). Free fatty acid was remained steady until day 9 and then increased to 2.07% at day 12 (Figure 45). It was shown that the storage time had the effect on these chemical properties of LNDC.

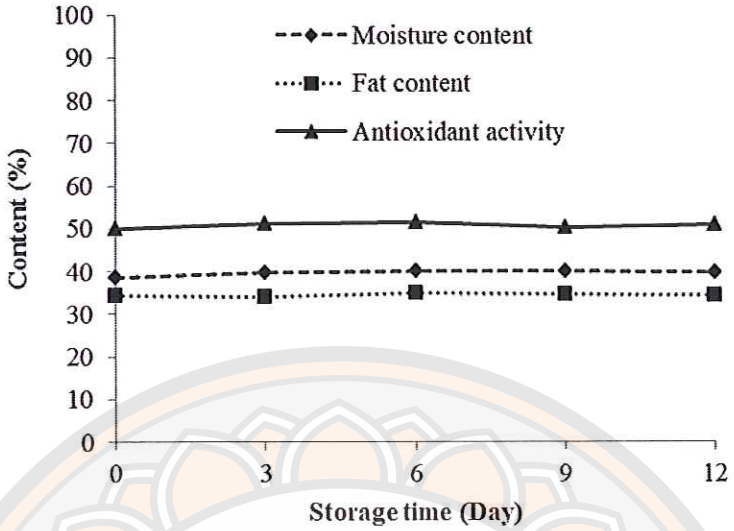

Figure 44 Moisture content, fat content and antioxidant activity of liquid non-dairy creamer during storage at 4±2° for 12 days

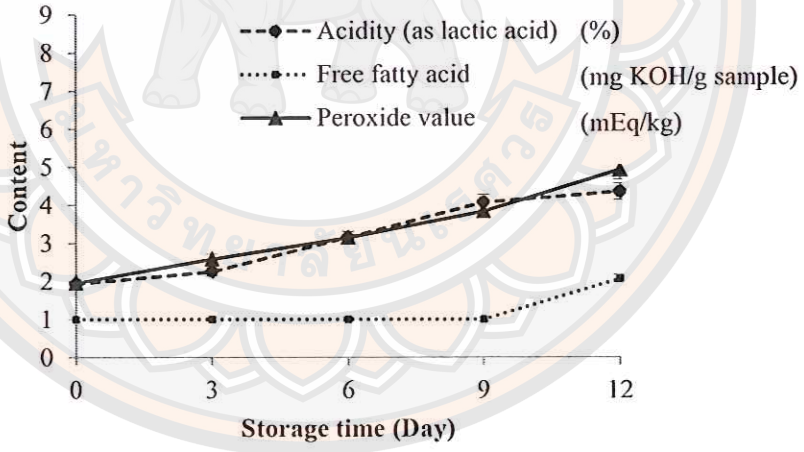

Figure 45 Acidity (as lactic acid), free fatty acid and peroxide value of liquid non-dairy creamer during storage at 4±2° for 12 days

When compared the antioxidant properties among mixed oil, LNDC at day 0 and day 12, it was shown that the value was decreased (Table 29). Because both γ-oryzanol and α-tocopherol were disintegrate during storage time. Total saturated fatty acids was increased from 10.64 to 14.15 (g/100 g) at 0 to 12 days. Total monounsaturated fatty acids and total polyunsaturated fatty acids at 12 days of storage

time were higher fatty acids than 0 day of storage time (11.29-13.74 g/100 g and 9.36-10.12 g/100 g, respectively). The fatty acid composition at day 12 was higher than that of day 0 because the time of storage affected to the fatty acid composition by the chemical mechanism, for example oxidative rancidity, photo oxidation or immigration of lipid. Polyunsaturated: Monounsaturated: Saturated fatty acids or PMS ratio of LNDC at day 0 was close to the recommended of USFDA than other samples. Trans fat content was not detected in the LNDC.

Table 29 Antioxidants, fatty acids, PMS ratio and trans fat content of liquid non-dairy creamer during storage at 4±2°C for 12 days

Properties	Storage Time (day)	
	0	12
Antioxidants		
γ-Oryzanol (mg/100g oil)	792.30	724.77
α-Tocopherol (mg/100g oil)	4.34	3.83
Fatty acid		
Saturated fatty acids		
C8:0 (g/100 g)	0.22	0.42
C10:0 (g/100 g)	0.24	0.36
C12:0 (g/100 g)	2.86	4.16
C14:0 (g/100 g)	0.95	1.15
C16:0 (g/100 g)	6.37	6.83
C18:0 (g/100 g)	0	1.23
Total saturated fatty acids	10.64	14.15
Unsaturated Fatty acids		
C18:1, cis-9 (g/100 g)	11.29	13.74
Total monounsaturated fatty acids	11.29	13.74
C18:2, cis (g/100g)	9.36	10.12
Total polyunsaturated fatty acids	9.36	10.12
PMS ratio	0.87:1.06:1	0.72:0.80:1
Trans fat	0	0

1.3 Microbiological properties of LNDC during storage time

The microbiological properties of LNDC during storage at 4±2°C for 12 days were shown in Table 30. Yeast and mold, coliform and *Escherichia coli* were not detectable in the LNDC. This result indicated that the LNDC was safe for consumption. However, the total viable count was detectable in LNDC that kept for 12 days (Table 30). The standard of total viable count was lower than log 10^3 cfu/mL. Therefore, according to this microbiological property, the shelf life of LNDC was between day 9-day 12. When compared with the commercial brand, the pasteurized milk had shelf life 1-2 weeks. Therefore, LNDC had similar shelf life to the commercial pasteurized milk.

Table 30 Microbiological properties of liquid non-dairy creamer during storage at 4±2°C for 12 days

Properties	Storage Time (day)				
	0	3	6	9	12
Total plate count (CFU/mL)	<10	<10	<10	2.00×10^2	6.36×10^4
Yeast and mold (CFU/mL)	<10	<10	<10	<10	<10
Coliform (MPN/mL)	<3	<3	<3	<3	<3
E. coli (MPN/mL)	<3	<3	<3	<3	<3
Lactic acid bacteria (MPN/mL)	<10	<10	<10	<10	<10

1.4 Sensory evaluation of LNDC during storage time

Figure 46 showed the result of liking score of LNDC that stored at 4±2°C, day 0 to day 9 because at day 12 the total plate count was detected at higher level than the standard level. For safety reason, the sensory evaluation was carried out between days 0-9. The liking scores of all attributes in each day were not significantly different ($P>0.05$). It was shown that the quality of the LNDC was not changed until day 12. The liking score of color of LNDC was the highest ($P\leq0.05$) (7; like moderately). All attributes had the liking scores higher than 6, which were considered acceptable.

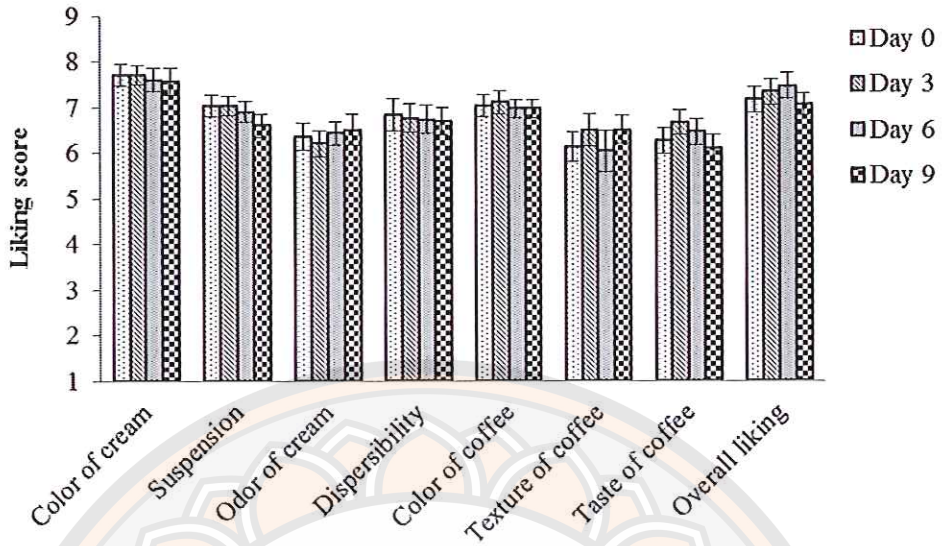

Figure 46 Sensory evaluation of LNDC during storage at 4±2°C for 12 days

2. Quality properties of PNDC during storage time

The quality of PNDC was estimated. After selection of the best formula from part 3 which was the ratio of HPKO: CRBO at 10:90. The PNDC samples were kept in aluminum foil at 3 g and kept in a hot air oven at 30 and 40°C for 80 days to determine the shelf life of the PNDC.

2.1 Physical properties of PNDC during storage time

Color (L*, a*, b* and ΔE)

The lightness (L*), a*, b* and ΔE (Figures 47-50) increased when increasing storage time. The L* of powder at both temperatures had the same trend. Wachiraya (2008) studied the production of powdered non-dairy creamer from CRBO and found that the range of L* was 92.77-94.12, a* was -2.53 - -2.31, b* was 9.55-12.43. These values were similar to the values of a* and b* but different in L* which was higher than the results in this present study (L* = 72.24-85.11).

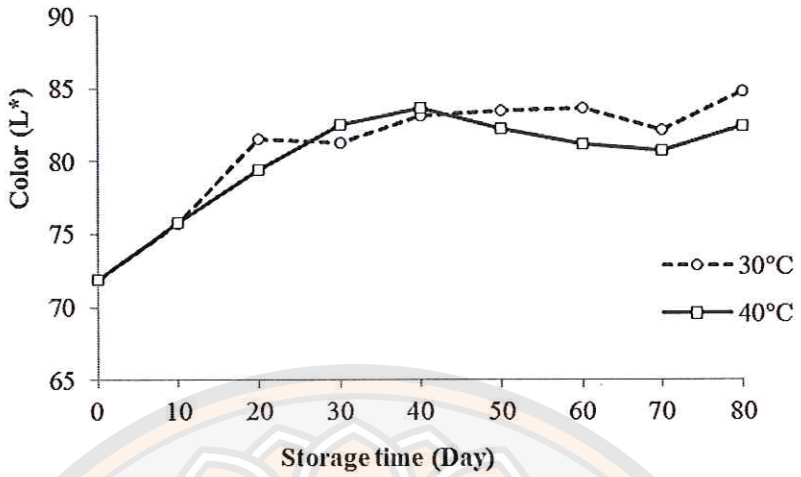

Figure 47 Color (L*) of PNDC during storage at 30 and 40°C for 80 days

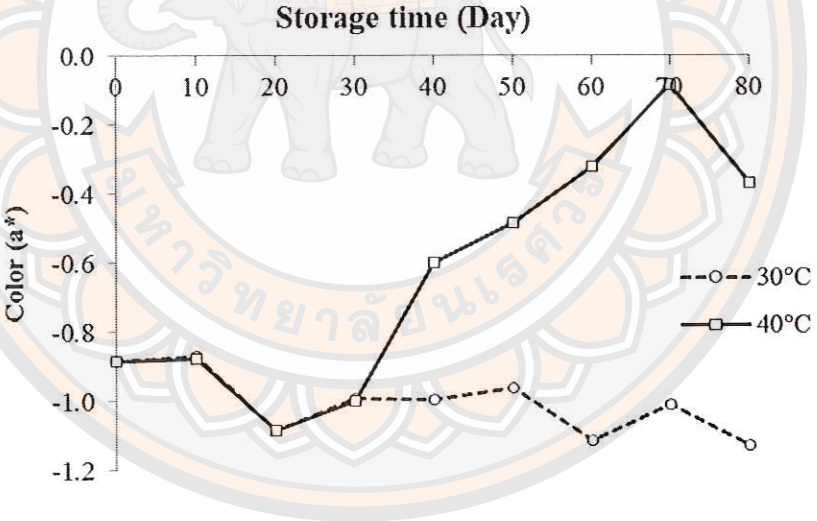

Figure 48 Color (a*) of PNDC during storage at 30 and 40°C for 80 days

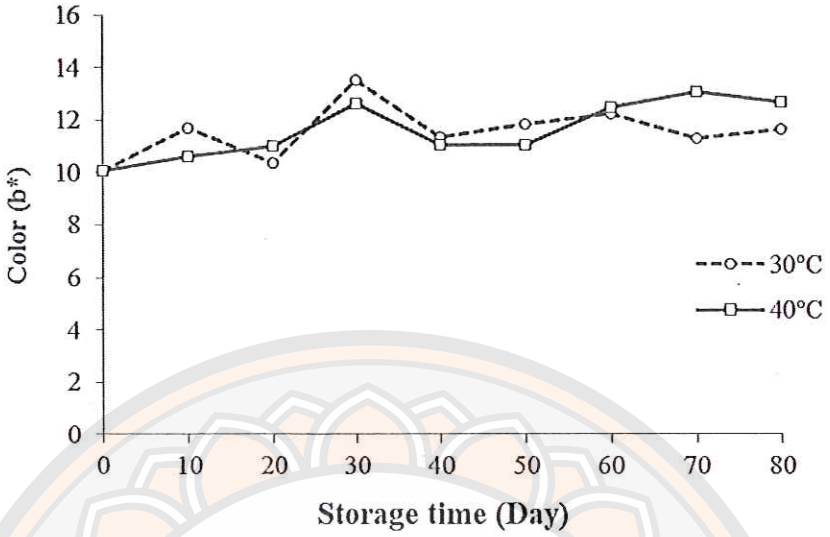

Figure 49 Color (b*) of PNDC during storage at 30 and 40°C for 80 days

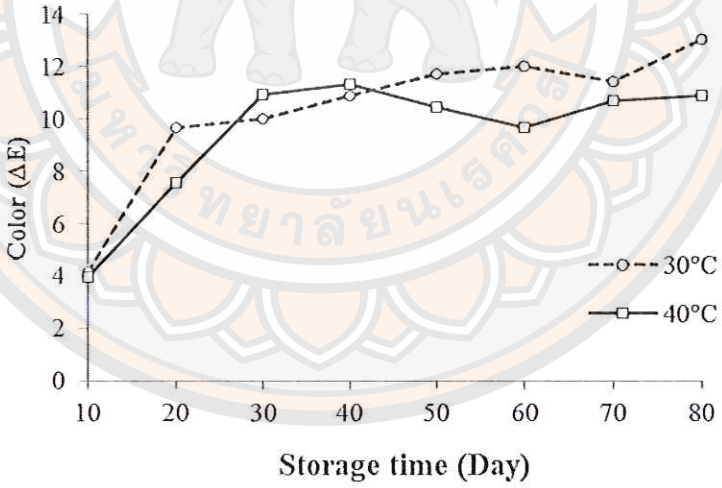

Figure 50 Color (ΔE) of PNDC during storage at 30 and 40°C for 80 days

Solubility

The solubility is the important quality of powder products. From Figure 51, the solubility of PNDC that stored at both temperatures was increased with storage time. This indicated that storage time and temperature affected the solubility of PNDC. The solubility properties of powder at 40°C were lower than at 30°C as it spent longer

time to dissolve. Wachiraya (2008) studied the production of powdered non-dairy creamer from CRBO and found that the solubility was 89-108 sec, which was longer than the results from this study.

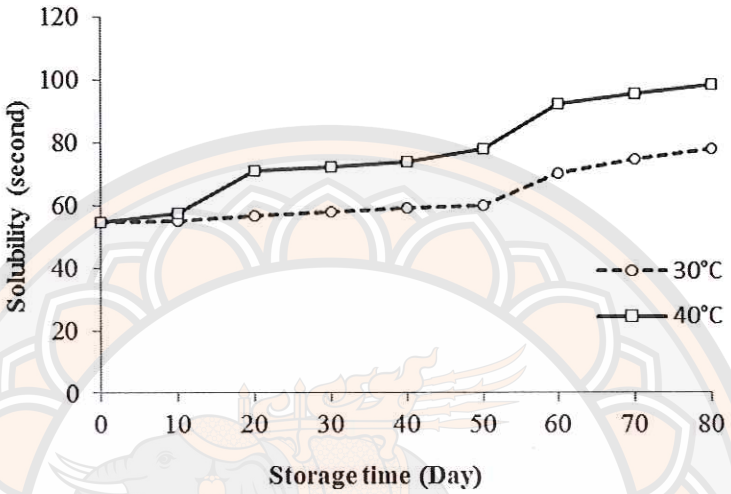

Figure 51 Solubility of PNDC during storage at 30 and 40°C for 80 days

Bulk Density

Bulk density of PNDC was shown in Figure 52. The density of powder during storage time at 30°C was lower than those stored at 40°C. It was indicated that the quality of powder at 30°C was better than at 40°C. On the other hand, the bulk density of PNDC (Figure 52) slightly decreased during storage at 30 and 40°C for 80 days. Naratip (1996) studied the development of non-dairy creamer from soy protein isolate and found that the bulk density was 0.37-0.55 g/cm^3. Patinya (2009) developed non-dairy creamer from coconut protein and found that the density was 0.34-0.36 g/cm^3. These findings were similar to the results of this study.

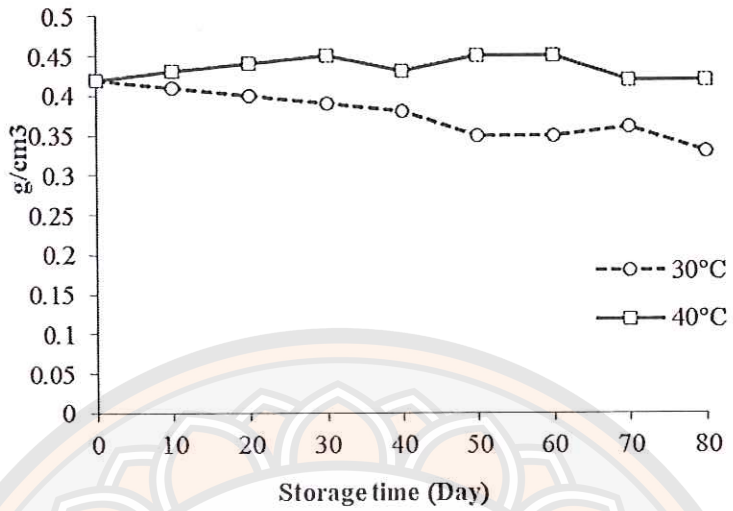

Figure 52 Bulk density of PNDC during storage at 30 and 40°C for 80 days

2.2 Chemical properties of PNDC during storage time

The changed in chemical properties of PNDC during storage at 30 and 40°C for 80 days were shown in Figures 53-59.

Moisture Content

Moisture content and water activity of PNDC were slightly decreased (Figures 53-54) with time of storage. The moisture content of PNDC stored at both temperatures was lower than the standard of PNDC (5%). Naratip (1996) reported that non-dairy creamer from soy protein isolate had the moisture content of 2.5-2.9%. Wachiraya (2008) reported that powdered non-dairy creamer from CRBO had moisture content of 4.20-4.78%. All these finding were in agreement with the results from this study.

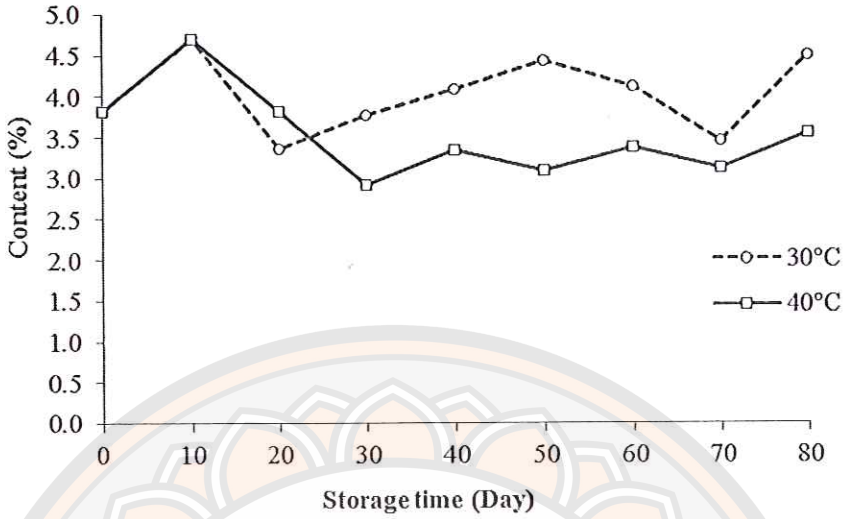

Figure 53 Moisture content of PNDC during storage at 30 and 40°C for 80 days

Water Activity

Water activity (a_w) is very important index for spray dried powder as it determines the shelf life of powder produced (Farimin and Nordin, 2009). Water activity is defined as the ratio of vapor pressure of water in a food system to vapor pressure of pure water at the same temperature. Water activity measures the activity of free water in a food system which is responsible for any biochemical reactions. Higher water activity indicates that there is more free water available for biochemical reactions and thus the shelf-life is shorter. Basically, food with a_w less than 0.6 is microbiologically stable. From Figure 54, the water activity of the PNDC produced was in the range of 0.20-0.30. This showed that PNDC that stored at both temperatures was microbiologically stable. Naratip (1996) reported that non-dairy creamer from soy protein isolate had a_w of 0.23-0.39 which were similar to the results of this study.

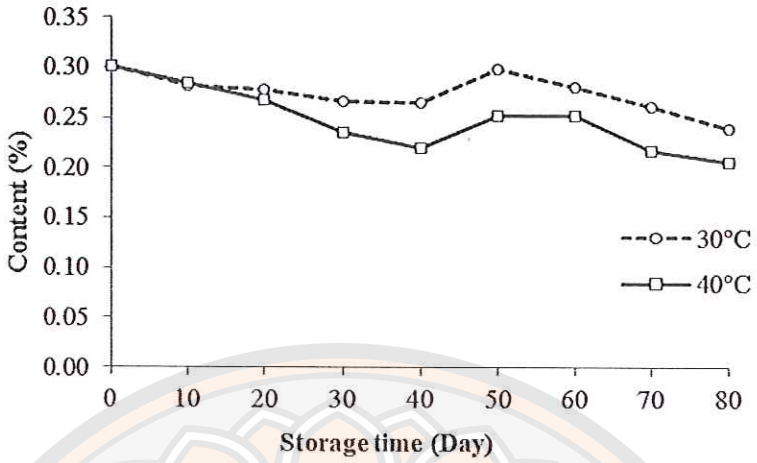

Figure 54 Water activity of PNDC during storage at 30 and 40°C for 80 days

Acidity (as lactic acid)

Both PNDC that stored at 30 and 40°C had an effect on the acidity. The acidity was not constant at 0-30 days. However after 30 days, they were slightly increased but not significant difference ($p>0.05$) at the same temperature (Figures 55). After 40 days at 40°C, PNDC had higher acidity than that of 30°C due to the temperature effect on the acidity of the PNDC.

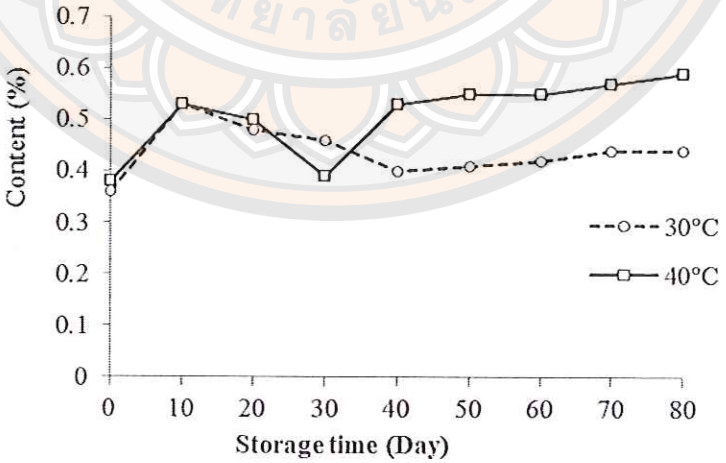

Figure 55 Acidity of PNDC during storage at 30 and 40°C for 80 days

Free Fatty Acid and Acid Value

Figure 56 showed free fatty acid content of PNDC during storage at 30 and 40°C for 80 days. From the results, free fatty acid content was increased with storage time. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides. Such reaction occurs by the action of lipase enzyme and it is an indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity, tissue damage). The source of the enzyme can be the tissue from which the oil or fat was extracted or it can be a contaminant from other cells including microorganisms. Besides FFA, hydrolysis of triglycerides produces glycerol.

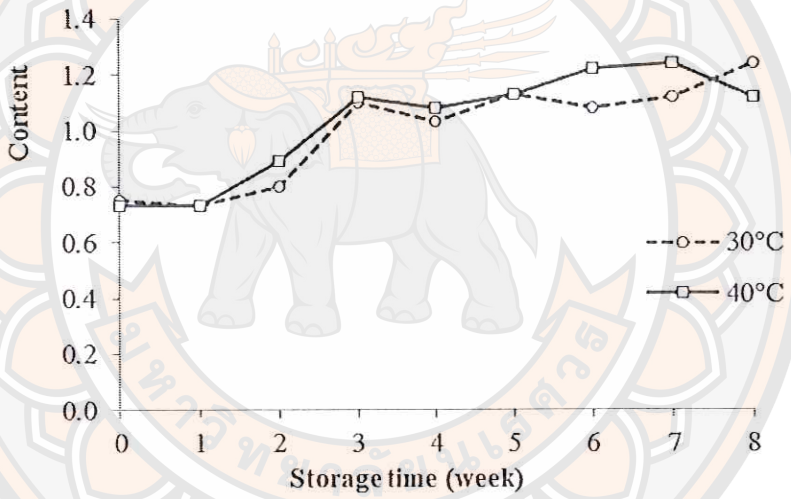

Figure 56 Free fatty acid content of PNDC during storage at 30 and 40°C for 80 days

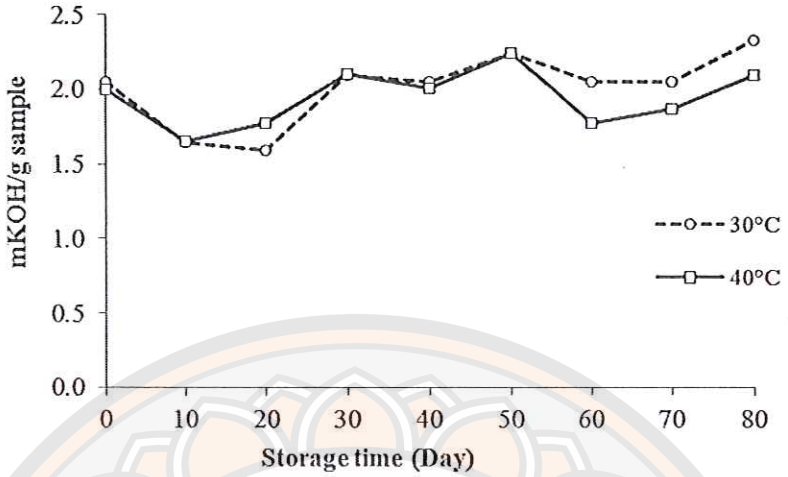

Figure 57 Acid value of PNDC during storage at 30 and 40°C for 80 days

Peroxide Value

Peroxide value significantly increased ($p \leq 0.05$) from 0 to 80 days (Figure 58). It could be shown that the storage temperature and time had effect on peroxide value of PNDC. The peroxide value is a good indicator of the quality of fat oil. Freshly refined fats should have hydroperoxide levels of less than 1 mEq/kg (Rosell, 1989). The limiting peroxide value specified by Joint FAO/WHO (1989) standards for refined oil is 10 mEq/kg. This study showed values of 1.4, 2.0, 2.2, 2.5, 2.8, 3.0, 3.1 and 3.2 mEq/kg for 10, 20, 30, 40, 50, 60, 70 and 80 days, respectively, indicating that it were conformed to the standard of oil (Figure 58).

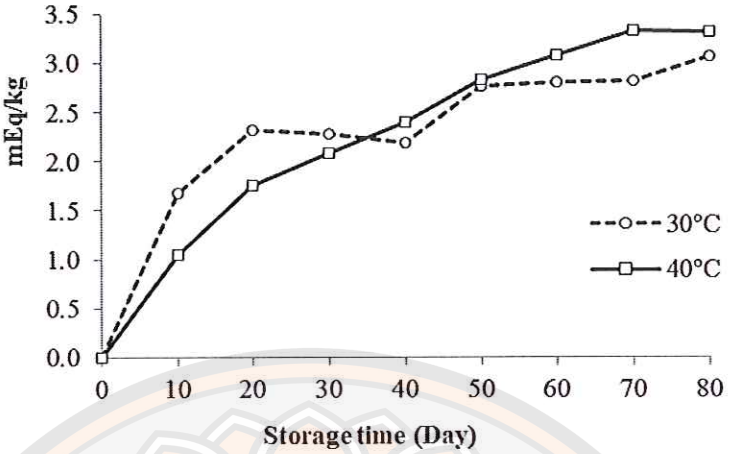

Figure 58 Peroxide value of PNDC during storage at 30 and 40°C for 80 days

Antioxidant Activity

Antioxidant activity of PNDC was shown in Figure 59. The antioxidant activity of PNDC decreased during storage at 30 and 40°C for 80 days. In both temperatures, scavenging of DPPH free radical were slowly decreased from 0 to 70 days and after 70 days the antioxidant activity (DPPH) was significantly decreased form 24% to 10%.

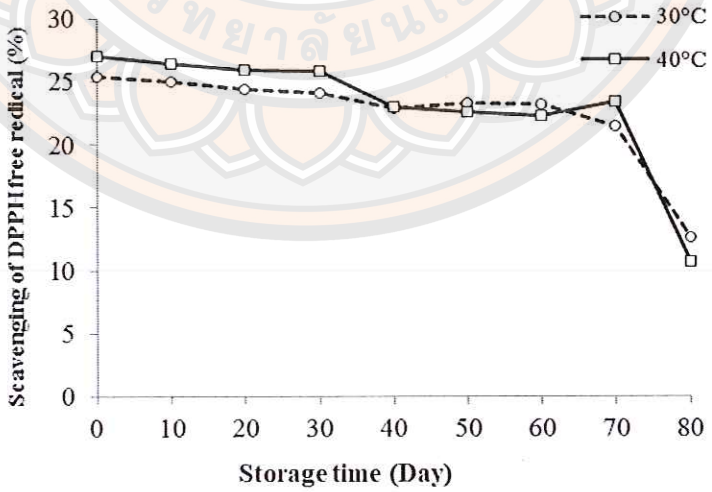

Figure 59 Antioxidant activity (DPPH) of PNDC during storage at 30 and 40°C for 80 days

2.3 Microbiological properties of PNDC during storage time

The microbiological properties of PNDC during storage at 30 and 40°C for 80 days were shown in Table 31. Total viable count, yeast and mold, coliform and *Escherichia coli* were not detectable in the PNDC. These results indicated that the PNDC was safe for consumption.

Table 31 Microbiological properties of PNDC during storage at 30 and 40°C for 80 days

Condition		Microorganisms			
		Total plate count (CFU/mL)	Yeast and mold (CFU/mL)	Coliform (MPN/mL)	*E. coli* (MPN/mL)
Day 0		<10	<10	<3	<3
Day 10	30°C	<10	<10	<3	<3
	40°C	<10	<10	<3	<3
Day 20	30°C	<10	<10	<3	<3
	40°C	<10	<10	<3	<3
Day 30	30°C	<10	<10	<3	<3
	40°C	<10	<10	<3	<3
Day 40	30°C	<10	<10	<3	<3
	40°C	<10	<10	<3	<3
Day 50	30°C	<10	<10	<3	<3
	40°C	<10	<10	<3	<3
Day 60	30°C	<10	<10	<3	<3
	40°C	<10	<10	<3	<3
Day 70	30°C	<10	<10	<3	<3
	40°C	<10	<10	<3	<3
Day 80	30°C	<10	<10	<3	<3
	40°C	<10	<10	<3	<3

2.4 Sensory evaluation of PNDC during storage time

Figure 59 showed the sensory evaluation results of PNDC during storage at 30°C and 40°C for 80 days. All of attributes in each week were significantly different ($P \leq 0.05$). It was shown that the quality of the product changed until days 70. The liking scores of days 10-20 were the highest ($p \leq 0.05$) whereas the average scores in days 70-80 was the lowest ($p \leq 0.05$). The scores of almost attributes of PNDC that kept at 30°C were higher than that of 40°C. The average score of all attributes was 7 (like moderately) which was considered acceptable.

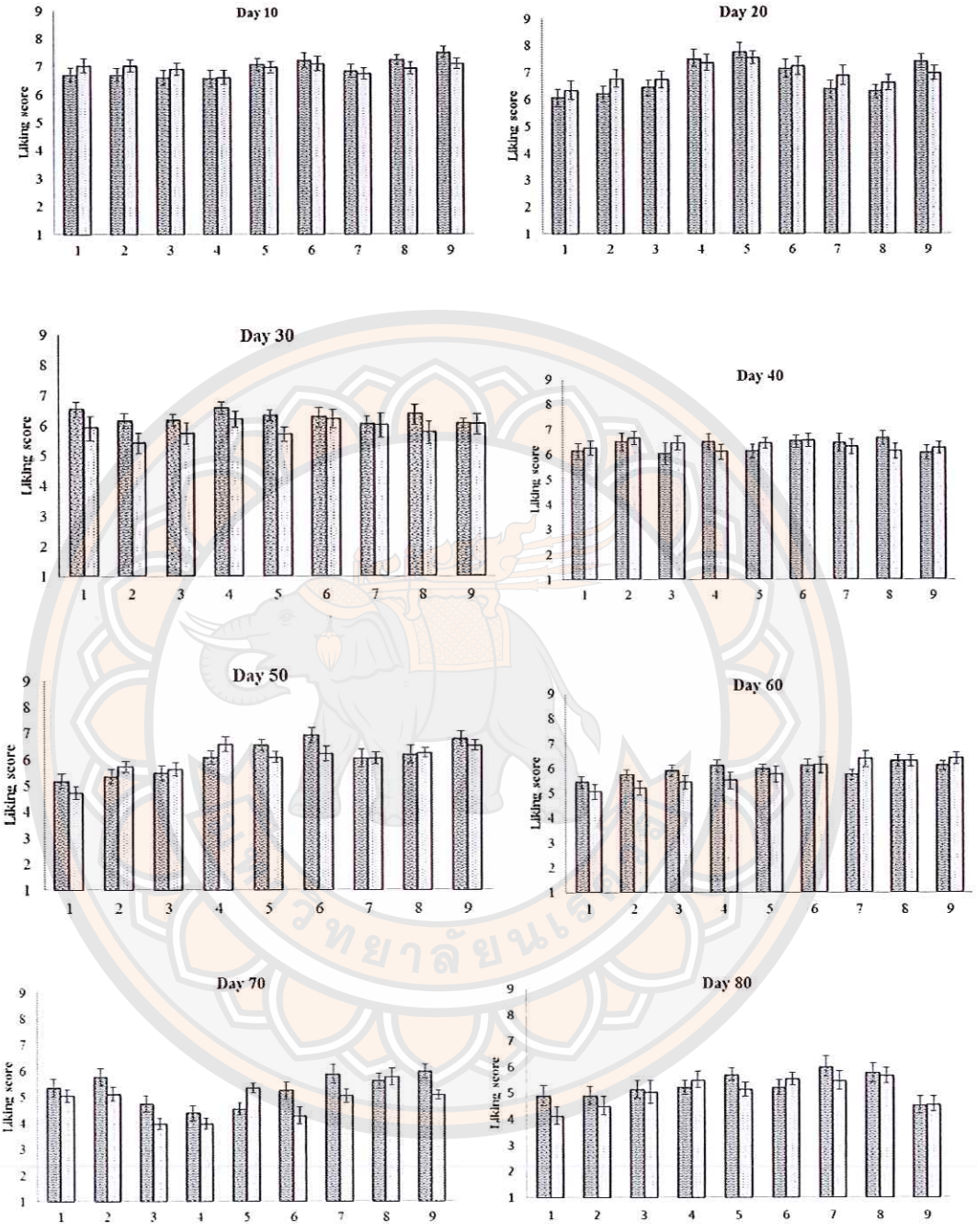

Figure 60 The liking score of PNDC storage at 30 (■) and 40 (□) °C for 80 days

Source: 1 = color of powder, 2 = powder suspension, 3 = rancidity,
4 = odor of powder, 5 = dispersibility, 6 = color of coffee,
7 = texture of coffee, 8 = taste of coffee and 9 = overall liking

Part 4 Marketing and consumer acceptance

This part aimed to find out whether the selected LNDC and PNDC from mixed HPKO and CRBO be accepted by the general consumers in terms of quality and price.

The selected LNDC and PNDC from mixed HPKO and CRBO were sensory tasted by 400 untrained consumers and using the 9-point Hedonic scale using the Central Location Test at Naresuan University. The details of consumers collected including sex, education, career, frequency of eating the products, willingness to purchase the products.

The questionnaires composed of 4 sections as following:

Section 1: General question about consumer

Section 2: Information about consumer behavior on non-dairy creamer

Section 3: Information about product development

Section 4: Characteristic of non-dairy creamer in consumer need

The results of the marketing and consumer acceptance of both LNDC and PNDC were as followed.

Section 1: General question about consumer

1. Gender

□ Male (122 people, 30.5%)

□ Female (278 people, 69.5%)

2. Age

□ Less than 20 (22 people, 5.5%)

□ 20-25 (143 people, 35.8%)

□ 26-35 (149 people, 37.3%)

□ 36-45 (49 people, 12.3%)

□ 46-55 (29 people, 7.3%)

□ More than 56 (8 people, 2%)

3. Occupation

□ School student (2 people, 30.5%)

□ College student (191 people, 30.5%)

□ Government officer (65 people, 30.5%)

□ Private employee (25 people, 30.5%)

□ State enterprise employee (28 people, 30.5%)

□ Agriculturist (6 people, 30.5%)

□ Laborer (10 people, 30.5%)

□ Housekeeper (4 people, 30.5%)

□ Others (66 people, 30.5%)

4. Salary

□ Less than 6,000 Baht/month (53 people, 30.5%)

□ 6,001-9,000 Baht/ (43 people, 30.5%)

□ 9,001-12,000 Baht/month (40 people, 30.5%)

□ 12,001-15,000 Baht/month (28 people, 30.5%)

□ 15,001-18,000 Baht/month (106 people, 30.5%)

□ More than 18,000 Baht/month (139 people, 30.5%)

5. Living style

□ Single (107 people, 26.8%)

□ Family (280 people, 70%)

□ Co-other (13 people, 3.3%)

6. Education

□ Primary school (88 people, 2%)

□ Secondary school (7 people, 1.8%)

□ High school (2 people, 0.5%)

□ Diploma (10 people, 2.5%)

□ Bachelor (217 people, 54.3%)

□ Graduate (156 people, 39%)

The consumer target was 400 people. The majority of consumers were female (278) and the number of male was 122. The age of consumer was divided into 6 groups. There were less than 20, 20-25, 26-35, 36-45, 46-55 and more than 56 years old. The numbers of consumer in each age group were 22, 143, 149, 49, 29 and 8, respectively. The occupation of consumers varied. However, most of them (47.8%) were students at the college and university. The majority of consumers had income higher than 15,000 Baht per month (61.3%). There were 3 types of living style which were stayed alone, stayed with family and stayed with others. The percentages were 26.8, 70.0 and 3.3, respectively. Diploma was the major group of graduated targeted consumers, following by graduate level.

Section 2: Information about consumer behavior on non-dairy creamer

7. Consume and never consume the product

□ Consume (69 people, 17.3%)

□ Never consume (331 people, 82.8%)

8. Like and dislike the product

□ Like (179 people, 44.8%)

□ Dislike (221 people, 55.3%)

9. Reason for consumption of the product

□ Taste (72 people, 18%)

□ Texture (23 people, 5.8%)

□ Coffee taste (122 people, 30.5%)

□ Odor (16 people, 4%)

□ Nutritional value (2 people, 0.5%)

□ Others (7 people, 1.8%)

10. Reason for not consumption of the product

□ Taste (47 people, 11.8%)

□ Expensive (18 people, 4.5%)

□ Odor (13 people, 3.3%)

□ Texture (16 people, 4%)

□ Others (40 people, 10%)

11. Frequency of consumption

□ 1 time/day (94 people, 23.5%)

□ 2 times/day (16 people, 4%)

□ 3 times/day (2 people,0.5%)

□ More than 3 times/day (2 people,0.5%)

□ 1 time/week (57 people, 14.3%)

□ 1 time/month (14 people, 3.5%)

□ 2-3 times/month (24 people, 6%)

□ Seldom (87 people, 21.8%)

12. Use the product in

□ Tea (38 people, 9.5%)

□ Coffee (182 people, 45.5%)

□ Cocoa (70 people, 17.5%)

□ Others (8 people, 2%)

13. Place for buying the product

□ Department store (Big C, Lotus, Makro) (181 people, 45.3%)

□ Convenient shop (7-11, 108 Shop) (78 people, 19.5%)

□ Grocery (13 people, 3.3%)

□ Others (24 people, 6%)

14. Size of the product normally buy

□ 1 g (28 people, 7%)

□ 5 g (84 people, 21%)

□ 10 g (66 people, 16.5%)

□ 15 g (52 people, 13%)

□ 20 g (20 people, 5%)

□ Others (36 people, 9%)

From the consumer acceptant test, 82.7% used to taste product but 17.3% never used the product. The amount of consumer who liked and disliked the product was 179 and 221 people, respectively. The taste and color of coffee were the buying reason by the consumers for non-dairy creamer (30.5%). The taste was a reason whether or not consume non-dairy creamer (11.8%). Frequency of consume non-dairy creamer was divided into 3 groups (always, sometimes and seldom). In the first group, 1 time/day had higher percentage (23.5%). The second group, 1 time/week was higher percentage (14.3%). The third group, 21.8% was seldom. Consumers preferred coffee (45.5%) mixed with non-dairy creamer, following by cocoa (17.5%) and tea (9.5%). Department store was favorite place to buy non-dairy creamer. The normal size of packaging with 5 g of non-dairy creamer showed higher percentage (21.0%) than other sizes.

Section 3: Information about product development

15. Do you know this product?

□ Known (228 people, 57%)

□ Unknown (172 people, 43%)

16. Have you ever consumed the product?

□ Consumed before (216 people, 54%)

□ Never (184 people, 46%)

17. If available, are you interested to buy this product?

□ Interest (252 people, 63%)

□ Not interest (148 people, 37%)

18. Why are you interested to buy this product?

□ New product (176 people, 44%)

□ Nutritional value (58 people, 14.4%)

□ Regular consume (33 people, 8.3%)

19. Why are you not interested to buy this product?

□ Scare (28 people, 7%)

□ Not drink tea or coffee (83 people, 20.8%)

□ Others (51 people, 12.8%)

There were 228 people (57%) knew about non-dairy creamer. The typical consumption of non-dairy creamer; 54% of consumers were consumed non-dairy creamer and 46% were never consumed it before. There were 252 consumers interested in LNDC and PNDC and 37% of consumers were not interested in both products. New product (44.0%) was the first interest reason following by nutrition value (14.4%). 8.3% of consumers were regular consumed non-dairy creamer. Not drink tea or coffee (20.8%) was the first reason of no interest following by scary (7.0%).

Section 4: Characteristic about non-dairy creamer in consumer need

20. The most important properties of the product

□ Taste (227 people, 56.8%)

□ Nutritional value (92 people, 23%)

□ Odor (22 people, 5.5%)

□ Color / appearance (16 people, 4%)

□ Texture (25 people, 6.3%)

□ Others (18 people, 4.5%)

21. Preferred type of packaging for liquid non-dairy creamer

□ Plastic bag (54 people, 13.5%)

□ Plastic cup (74 people, 18.5%)

□ Glass bottle (184 people, 46%)

□ Aluminum foil (72 people, 18%)

□ Others (16 people, 4%)

22. Preferred type of packaging for powdered non-dairy creamer

□ Plastic bag (94 people, 23.5%)

□ Plastic cup (55 people, 13.8%)

□ Glass bottle (92 people, 23%)

□ Aluminum foil (149 people, 37.3%)

□ Others (10 people, 2.5%)

23. Appropriated serving size for liquid non-dairy creamer

□ 15 ml/ pack in plastic cup (130 people, 32.5%)

□ 20 ml/ pack in plastic cup (88 people, 22%)

□ 100 ml/ pack in plastic bottle (55 people, 13.8%)

□ 200 ml/ plastic bottle (23 people, 5.8%)

□ 100 ml/ pack in glass bottle (64 people, 16%)

□ 200 ml/ pack in glass bottle (20 people, 5%)

□ Others (20 people, 5%)

24. Appropriated serving size for powdered non-dairy creamer

□ 3 g/50 packs in plastic bag (152 people, 38%)

□ 3 g/100 packs in plastic bag (78 people, 19.5%)

□ 75 g/ pack in aluminum foil (38 people, 9.5%)

□ 100 g/ pack in aluminum foil (61 people, 15.3%)

□ 200 g/ pack in aluminum foil (32 people, 8%)

□ 400 g/ pack in plastic bottle (19 people, 4.8%)

□ Others (20 people, 5%)

25. Appropriated price you prefer in liquid non-dairy creamer

□ 1-5 Baht (77 people, 19.3%)

□ 6-10 Baht (139 people, 34.8%)

□ 11-30 Baht (53 people, 13.3%)

□ 31-60 Baht (68 people, 17%)

□ 61-90 Baht (43 people, 10.8%)

□ 91-120 Baht (13 people, 3.3%)

□ Other (7 people, 1.8%)

26. Appropriated price you prefer in powdered non-dairy creamer

□ 15-30 Baht (52 people, 13%)

□ 31-50 Baht (27 people, 6.8%)

□ 51-60 Baht (142 people, 35.5%)

□ 61-70 Baht (96 people, 24%)

□ 71-80 Baht (54 people, 13.5%)

□ Others (29 people, 7.3%)

27. The most important factor for buying non-dairy creamer

□ Price (186 people, 46.5%)

□ Nutritional value (134 people, 33.5%)

□ Type of packaging (22 people, 5.5%)

□ Amount per unit (5 people, 1.3%)

□ Selling place (4 people, 1%)

□ Convenient (31 people, 7.8%)

□ Others (18 people, 4.5%)

This section was involved characteristic LNDC and PNDC. There were including packaging, weight and price of product. The important factor of non-dairy creamer was taste (54.0%), nutrition value (23.0%), texture (6.3%) and odor (5.5%), respectively. 43.5% of consumers were chosen glass bottle for LNDC package following by plastic cup and aluminum foil (18.5 and 18.0%, respectively). Consumers were

chosen aluminum foil for PNDC package (34.3%) following by plastic bag and glass bottle (23.5 and 23.0%, respectively). Consumers were chosen 15 ml/plastic cup (25.5%). The other weighting per unit for LNDC was 20 ml/plastic cup (22.0%). Consumers were chosen 3 g/plastic cup (32.0%) which was the highest score. The other weighting per unit for PNDC was 3 g/100 plastic bag (19.5%). The suitable price of LNDC was 6-10 baht (29.3%). The suitable price of PNDC was 30-40 baht (30.0%). The most important factor that effecting decision were price (42.5%) and nutrition value (35.5%).

The liking score of LNDC and PNDC for consumer acceptance by 400 untrained panelists were shown in Figures 61 and 62, respectively. Color of coffee of LNDC had the highest score (8.8) following by texture of coffee (8.0) and overall liking (7.9) but odor of cream had the lowest score (6.6) (Figure 60).

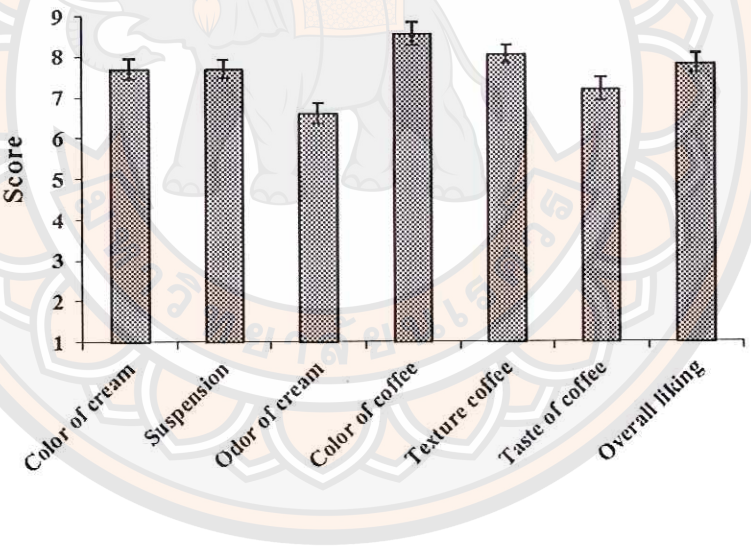

Figure 61 The liking score of LNDC for consumer acceptance by 400 untrained panelists

Color of coffee of PNDC had the highest score (8.1) but not significant different from the color of powder (7.8) and overall liking (7.9). However odor of cream had the lowest score too (6.3) (Figure 62). Finally, the consumer was acceptance in LNDC and PNDC.

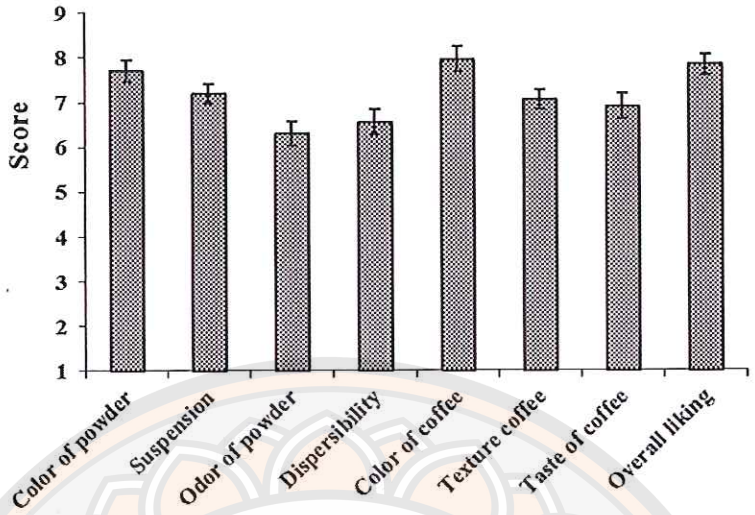

Figure 62 The liking score of PNDC for consumer acceptance by 400 untrained panelists

CHAPTER V

CONCLUSION

This study illustrated that mixtures of HPKO and CRBO had various physical and chemical properties in terms of viscosity, color, trans fat, fatty acid composition, γ-oryzanol, α-tocopherol and antioxidant activity. The HPKO: CRBO mixtures of 80-100:20-0 contained trans fat, therefore, they should not be selected for further utilization in food products. Substitution of HPKO by CRBO from 30-100% would be suitable for food applications in terms of nutrition and health. In this study, the suitable mixing ratio of HPKO: CRBO for LNDC and PNDC were 20:80 and 10:90, respectively.

The shelf life of the pasteurized LNDC was between 9-11 days. The production process was pasteurized the LNDC at 75°C for 15 seconds, kept in the polypropylene plastic bottle and stored in the refrigerator at 4±2°C. The pasteurized LNDC was cream in color. The antioxidant was still maintained in the pasteurized LNDC. The main fatty acids in CRBO were also present in the pasteurized LNDC. The pasteurized LNDC was safe in term of microbiological property. The panelist liking score of LNDC was about 7-8 (like moderately-like very much). The pasteurized LNDC had no trans fat. For PNDC, the condition of shelf life determination was keeping in hot air oven at 30 and 40°C for 80 days. The powder was kept in polypropylene plastic bag. The panelist liking score was about 7-8 (like moderately-like very much).

The cost analysis of products was calculated per one serving size. One unit of LNDC was 250 milliliter in polypropylene plastic bottle with a serving size of 15 mL. The PNDC was packed at 3 g (serving size) in polypropylene plastic bag. The price per serving size of LNDC and PNDC was 4.44 and 2.91 Baht, respectively.

The marketing and consumer acceptance of both LNDC and PNDC were tested by 400 untrained panelists. The majority group of consumer was female (69.5%). The ages were between 20-35 years old (72.8%). Most of them graduated with Bachelor degree (54%) and the salary was higher than 15,000 Baht per month

(61.3%). They lived with family (69.8%) and status of occupation was mostly college students (47.8%). The coffee taste was the first reason for consumption of non-dairy creamer. 63% of them were interested to buy non-dairy creamer because it is a new product. The appropriate serving size LNDC was 15 ml/ pack in plastic cup with the price of 6-10 Baht, respectively. Whereas for the PNDC was 3 g/50 packs in plastic bag with the price of 15-30 Baht. The most important factors for buying LNDC and PNDC were price and nutritional value of the products.

Recommendations

1. The LNDC and PNDC should be tasted with other powders such as tea, cocoa powder or ovaltine (related product).

2. Researcher should determine the properties of water as the ingredient before using in laboratory because hard water affects the quality of LNDC and PNDC.

3. Remove RBO odor is necessary because it strongly affects the consumer acceptance.

4. The present of scum in PNDC after mixing with coffee should be improved because it strongly affects the consumer acceptance.

5. Solubility and dispersibility of PNDC should be improved because it strongly affects the consumer acceptance.

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APPENDIX

APPENDIX A THAI INDUSTRIAL STANDARD

ประกาศกระทรวงสาธารณสุข

(ฉบับที่ 208) พ.ศ.2543

เรื่อง ครีม

โดยที่เป็นการสมควรปรับปรุงประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง ครีม

อาศัยอำนาจตามความในมาตรา 5 และมาตรา 6(3)(4)(5)(6)(7) และ (10) แห่งพระราชบัญญัติอาหาร พ.ศ.2522 อันเป็นพระราชบัญญัติที่มีบทบัญญัติบางประการเกี่ยวกับการจำกัดสิทธิและ เสรีภาพของบุคคล ซึ่งมาตรา 29 ประกอบกับมาตรา 35 มาตรา 48 และมาตรา 50 ของรัฐธรรมนูญแห่ง ราชอาณาจักรไทยบัญญัติให้กระทำได้โดยอาศัยอำนาจตามบทบัญญัติแห่งกฎหมาย รัฐมนตรีว่าการกระทรวงสาธารณสุขออกประกาศไว้ ดังต่อไปนี้

ข้อ 1 ให้ยกเลิกประกาศกระทรวงสาธารณสุข ฉบับที่ 49 (พ.ศ.2523) เรื่อง ครีม ลงวันที่ 4 มีนาคม พ.ศ.2523

ข้อ 2 ให้ครีมเป็นอาหารที่กำหนดคุณภาพหรือมาตรฐาน

ข้อ 3 ในประกาศนี้

"ครีม" หมายความว่า ครีมแท้ ครีมผสม และครีมเทียม

"ครีมแท้" หมายความว่า ผลิตภัณฑ์ที่แยกได้จากนม โดยกรรมวิธีต่าง ๆ และมีมันเนยเป็นส่วนประกอบที่สำคัญ

"ครีมผสม" หมายความว่า ครีมแท้ที่มีไขมันอื่นเป็นส่วนผสมอยู่ด้วย

"ครีมเทียม" หมายความว่า ผลิตภัณฑ์ที่มิได้ทำจากนมและมีไขมันอื่นนอกจากมันเนยเป็นส่วนประกอบที่สำคัญ

หรือครีมที่มีมันเนยผสมอยู่น้อยกว่าร้อยละ 30 ของไขมันทั้งหมด

"ครีมเปรี้ยว" หมายความว่า ครีมที่หมักด้วยจุลินทรีย์ที่ไม่ทำให้เกิดโรค หรือที่ไม่ทำให้เกิดพิษ และมีจุลินทรีย์ดังกล่าวที่มีชีวิตคงเหลืออยู่จากกรรมวิธีการหมักนั้น

ข้อ 4 ครีมแบ่งออกเป็น 5 ชนิด ดังต่อไปนี้

(1) ครีมพร่องมันเนย (Half cream)

(2) ครีมธรรมดา (Cream หรือ Single cream)

(3) วิปปิ้งครีม (Whipping cream)

(4) ดับเบิ้ลครีม (Double cream หรือ Heavy cream หรือ Thick cream)

(5) ครีมเปรี้ยว (Sour cream)

ข้อ 5 ครีมแท้ ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) ทำจากนม

(2) มีมันเนย ดังต่อไปนี้

(2.1) ไม่น้อยกว่าร้อยละ 10 และไม่ถึงร้อยละ 18 ของน้ำหนัก สำหรับครีมแท้ชนิดพร่องมันเนย

(2.2) ไม่น้อยกว่าร้อยละ 18 ของน้ำหนัก สำหรับครีมแท้ชนิดธรรมดา

(2.3) ไม่น้อยกว่าร้อยละ 28 ของน้ำหนัก สำหรับครีมแท้ชนิดวิปปิ้งครีม

(2.4) ไม่น้อยกว่าร้อยละ 36 ของน้ำหนัก สำหรับครีมแท้ชนิดดับเบิ้ลครีม

(2.5) ไม่น้อยกว่าร้อยละ 10 ของน้ำหนัก สำหรับครีมแท้ชนิดครีมเปรี้ยว

(3) มีความเป็นกรด คำนวณเป็นกรดแลคติคได้ไม่เกินร้อยละ 0.2 ของน้ำหนัก นอกจากครีมเปรี้ยว

(4) ตรวจไม่พบแบคทีเรียชนิด อี.โคไล (*Escherichia coli*) ในอาหาร 0.01 กรัม

(5) ไม่มีกลิ่นหืน

(6) ไม่มีวัตถุกันเสีย

(7) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค

(8) ไม่มีสารเป็นพิษจากจุลินทรีย์ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ

(9) ใช้ก๊าซที่ไม่เป็นพิษหรือเป็นอันตรายต่อสุขภาพ ในกรรมวิธีการผลิตวิปปิ้งครีม

ข้อ 6 ครีมแท้ที่ทำให้แห้ง ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) ทำจากนม

(2) มีลักษณะเป็นผง ไม่เกาะเป็นก้อน หรือมีลักษณะตามรูปลักษณะนั้น

(3) มีมันเนยไม่น้อยกว่าร้อยละ 42 ของน้ำหนัก

(4) มีความชื้นไม่เกินร้อยละ 5 ของน้ำหนัก

(5) ตรวจพบแบคทีเรียไม่เกิน 100,000 ในอาหาร 1 กรัม

(6) ไม่มีกลิ่นหืน

(7) ไม่มีวัตถุกันเสีย

(8) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค

(9) ไม่มีสารเป็นพิษจากจุลินทรีย์ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ

ข้อ 7 ครีมผสม ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) มีมันเนยผสมอยู่ไม่น้อยกว่าร้อยละ 30 ของไขมันทั้งหมด และ

(1.1) มีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 10 และไม่ถึงร้อยละ 18 ของน้ำหนักสำหรับครีมผสมชนิดพร่องมันเนย

(1.2) มีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 18 ของน้ำหนัก สำหรับครีมผสมชนิดธรรมดา

(1.3) มีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 28 ของน้ำหนัก สำหรับครีมผสมชนิดวิปปิ้งครีม

(1.4) มีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 36 ของน้ำหนัก สำหรับครีมผสมชนิดดับเบิ้ลครีม

(2) มีความเป็นกรด คำนวณเป็นกรดแลคติคได้ไม่เกินร้อยละ 0.2 ของน้ำหนัก

(3) ตรวจไม่พบแบคทีเรียชนิด อี.โคไล (*Escherichia coli*) ในอาหาร 0.01 กรัม

(4) ไม่มีกลิ่นหืน

(5) ไม่มีวัตถุกันเสีย

(6) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค

(7) ไม่มีสารเป็นพิษจากจุลินทรีย์ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ

(8) ใช้ก๊าซที่ไม่เป็นพิษหรืออันตรายต่อสุขภาพ ในกรรมวิธีการผลิตครีมผสมชนิดวิปปิ้งครีม

ข้อ 8 ครีมผสมที่ทำให้แห้ง ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) มีลักษณะเป็นผง ไม่เกาะเป็นก้อน หรือมีลักษณะตามรูปลักษณะนั้น

(2) มีไขมันไม่น้อยกว่าร้อยละ 40 ของน้ำหนัก

(3) มีความชื้นไม่เกินร้อยละ 5 ของน้ำหนัก

(4) ตรวจพบแบคทีเรียไม่เกิน 100,000 ในอาหาร 1 กรัม

(5) ไม่มีกลิ่นหืน

(6) ไม่มีวัตถุกันเสีย

(7) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค

(8) ไม่มีสารเป็นพิษจากจุลินทรีย์ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ

ข้อ 9 ครีมเทียม ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) มีไขมัน ดังต่อไปนี้

(1.1) ไม่น้อยกว่าร้อยละ 10 และไม่ถึงร้อยละ 18 ของน้ำหนัก สำหรับครีมเทียมชนิดพร่องไขมัน

(1.2) ไม่น้อยกว่าร้อยละ 18 ของน้ำหนัก สำหรับครีมเทียมชนิดธรรมดา

(1.3) ไม่น้อยกว่าร้อยละ 28 ของน้ำหนัก สำหรับครีมเทียมชนิดวิปปิ้งครีม

(1.4) ไม่น้อยกว่าร้อยละ 36 ของน้ำหนัก สำหรับครีมเทียมชนิดดับเบิ้ลครีม

(2) มีความเป็นกรดคำนวณเป็นกรดแลคติคได้ไม่เกินร้อยละ 0.2 ของน้ำหนัก

(3) ตรวจไม่พบแบคทีเรียชนิด อี.โคไล (*Escherichia coli*) ในอาหาร 0.01 กรัม

(4) ไม่มีกลิ่นหืน

(5) ไม่มีวัตถุกันเสีย

(6) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค

(7) ไม่มีสารเป็นพิษจากจุลินทรีย์ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ

(8) ใช้ก๊าซที่ไม่เป็นพิษหรืออันตรายต่อสุขภาพในกรรมวิธีการผลิตครีมเทียมชนิดวิปปิ้งครีม

ข้อ 10 ครีมเทียมที่ทำให้แห้ง ต้องมีคุณภาพหรือมาตรฐาน ดังต่อไปนี้

(1) มีลักษณะเป็นผง ไม่เกาะเป็นก้อน หรือมีลักษณะตามรูปลักษณะนั้น

(2) มีไขมันทั้งหมดไม่น้อยกว่าร้อยละ 30 ของน้ำหนัก

(3) มีความชื้นไม่เกินร้อยละ 5 ของน้ำหนัก

(4) ตรวจพบแบคทีเรียไม่เกิน 100,000 ในอาหาร 1 กรัม

(5) ไม่มีกลิ่นหืน

(6) ไม่มีวัตถุกันเสีย

(7) ไม่มีจุลินทรีย์ที่ทำให้เกิดโรค

(8) ไม่มีสารเป็นพิษจากจุลินทรีย์ในปริมาณที่อาจเป็นอันตรายต่อสุขภาพ

ข้อ 11 การใช้วัตถุเจือปนอาหาร ให้ปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่องวัตถุเจือปนอาหาร

ข้อ 12 ผู้ผลิตหรือผู้นำเข้าครีมเพื่อจำหน่าย ต้องปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง วิธีการผลิต เครื่องมือเครื่องใช้ในการผลิต และการเก็บรักษาอาหาร

ข้อ 13 การใช้ภาชนะบรรจุครีม ให้ปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง ภาชนะบรรจุ

ข้อ 14 การแสดงฉลากของครีม ให้ปฏิบัติตามประกาศกระทรวงสาธารณสุขว่าด้วยเรื่อง ฉลาก

ข้อ 15 ให้ใบสำคัญการขึ้นทะเบียนตำรับอาหารหรือใบสำคัญการใช้ฉลากอาหารตามประกาศกระทรวงสาธารณสุข ฉบับที่ 49 (พ.ศ.2523) เรื่อง ครีม ลงวันที่ 4 มีนาคม พ.ศ.2523 ซึ่งออกให้ก่อนวันที่ประกาศนี้ใช้บังคับยังคงใช้ต่อไปได้อีกสองปี นับตั้งแต่วันที่ประกาศนี้ใช้บังคับ

ข้อ 16 ให้ผู้ผลิต ผู้นำเข้าครีมที่ได้รับอนุญาตอยู่ก่อนวันที่ประกาศนี้ใช้บังคับ ยื่นคำขอรับเลขสารบบอาหารภายในหนึ่งปี นับแต่วันที่ประกาศนี้ใช้บังคับ เมื่อยื่นคำขอดังกล่าวแล้วให้ได้รับการผ่อนผันการปฏิบัติตามข้อ 12 ภายในสองปี นับแต่วันที่ประกาศนี้ใช้บังคับ และให้คงใช้ฉลากเดิมที่เหลืออยู่ต่อไปจนกว่าจะหมดแต่ต้องไม่เกินสองปี นับแต่วันที่ประกาศนี้ใช้บังคับ

ข้อ 17 ประกาศนี้ ให้ใช้บังคับเมื่อพ้นกำหนดหนึ่งร้อยแปดสิบวันนับแต่วันถัดจากวันประกาศในราชกิจจานุเบกษาเป็นต้นไป

ประกาศ ณ วันที่ 19 กันยายน พ.ศ.2543

กร ทัพพะรังสี

รัฐมนตรีว่าการกระทรวงสาธารณสุข

(ราชกิจจานุเบกษาฉบับประกาศทั่วไป เล่ม 118 ตอนพิเศษ 6 ง. ลงวันที่ 24 มกราคม พ.ศ.2544)

APPENDIX B THE PROTOCOL WAS APPROVED BY THE ETHICAL REVIEW COMMITTEE OF NARESUAN UNIVERSITY

ประกาศบัณฑิตวิทยาลัย มหาวิทยาลัยนเรศวร
เรื่อง อนุมัติให้นิสิตระดับปริญญาเอกดำเนินการทำวิจัย
ครั้งที่ 97/2555

.....

บัณฑิตวิทยาลัยอนุมัติให้ นายคุณากร ชัติศรี รหัสประจำตัว 53040348 นิสิตระดับปริญญาเอก หลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต สาขาวิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร ดำเนินการทำวิจัย ตามโครงร่างวิทยานิพนธ์ที่เสนอ

เรื่อง ภาษาไทย "อัตราส่วนที่เหมาะสมระหว่างไขมันแข็ง (ปาล์มโอเลอีน) และน้ำมันรำข้าวบีบเย็น ในกระบวนการผลิตครีมเทียมเหลว และครีมเทียมผง"

ภาษาอังกฤษ "The optimum mixing ratio between hard fat (palm olein) and cold pressed rice bran oil for production of liquid and powdered non – dairy creamer"

โดยมี ผู้ช่วยศาสตราจารย์ ดร.เหรียญทอง สิงห์จานุสงค์ เป็นประธานที่ปรึกษาวิทยานิพนธ์

จึงประกาศมาให้ทราบโดยทั่วกัน

ประกาศ ณ วันที่ 8 พฤศจิกายน พ.ศ. 2555

(ผู้ช่วยศาสตราจารย์ ดร.คนึงนิจ ภู่พัฒน์วิบูลย์)
คณบดีบัณฑิตวิทยาลัย มหาวิทยาลัยนเรศวร

เรียน คณบดี
- เพื่อโปรดทราบ
- ให้แจ้งอาจารย์ที่ปรึกษาวิทยานิพนธ์
1. ผศ.ดร. เหรียญทอง สิงห์จานุสงค์
2. นายคุณากร ชัติศรี

12 พ.ย. 55

APPENDIX C PARTICIPANT INFORMATION SHEET

"This participant information sheet is in Thai, Please contract the authors for English version"

ข้อมูลและคำแนะนำสำหรับผู้ป่วยหรืออาสาสมัครผู้เข้าร่วมโครงการวิจัย

(Participant Information Sheet)

1. **ชื่อโครงการศึกษา** อัตราส่วนที่เหมาะสมระหว่างไขมันแข็ง (ปาล์มโอเลอีน) และน้ำมันรำข้าวในกระบวนการผลิตครีมเทียมเหลวและครีมเทียมผง

2. **วัตถุประสงค์ของการวิจัย** (การศึกษานี้เกี่ยวกับเรื่องอะไร)

วัตถุประสงค์หลักของงานวิจัยเพื่อหาอัตราส่วนที่เหมาะสมระหว่างไขมันแข็ง (ปาล์มโอเลอีน) และน้ำมันรำข้าวบีบเย็น สูตรและกระบวนการผลิตครีมเทียมเหลวจากไขมันแข็งและน้ำมันรำข้าว

วัตถุประสงค์รองของงานวิจัย

1. ศึกษาคุณสมบัติของไขมันแข็ง (ปาล์มโอเลอีน) น้ำมันรำข้าวและน้ำมันผสม

2. พัฒนาสูตรและกระบวนการผลิตครีมเทียมเหลวและครีมเทียมผงจากไขมันแข็ง (ปาล์มโอเลอีน) และน้ำมันรำข้าว

3. ประเมินอายุการเก็บรักษาของครีมเทียมเหลวและครีมเทียมผงจากไขมันแข็ง (ปาล์มโอเลอีน) และน้ำมันรำข้าว

4. วิเคราะห์ต้นทุนการผลิต การตลาดและการยอมรับของผู้บริโภคต่อครีมเทียมเหลวและครีมเทียมผงจากไขมันแข็ง (ปาล์มโอเลอีน) และน้ำมันรำข้าว

3. **อธิบายเหตุผลที่อาสาสมัครได้รับเชิญเข้าร่วมโครงการ**

เนื่องจากอาสาสมัครเป็นผู้ที่บริโภคกาแฟเป็นประจำซึ่งสามารถรับรู้รสชาติของกาแฟและคุณลักษณะของกาแฟที่ดีและสามารถให้ข้อเสนอแนะที่เป็นประโยชน์ต่อการพัฒนาสูตรครีมเทียม ที่จะให้กาแฟมีรสชาติกลมกล่อมและคุณลักษณะที่ดีได้ ซึ่งทั้งนี้อาสาสมัครจะต้องมีอายุไม่ต่ำกว่า 20 ปี และไม่มีโรคประจำตัวที่มีสาเหตุจากการดื่มกาแฟ

4. **ขั้นตอนของการวิจัย วิธีรวบรวมข้อมูล รวมถึงการทดลองใดๆ และการนำเครื่องมือเข้าไปในร่างกาย** (invasive procedures)

งานวิจัยนี้ได้แบ่งออกเป็น 5 ขั้นตอน ดังนี้

ขั้นตอนที่ 1 การศึกษาคุณสมบัติของน้ำมันปาล์มโอเลอีนและน้ำมันรำข้าวที่นำมาผลิตครีมเทียม

ขั้นตอนที่ 2 การพัฒนาสูตรสำหรับครีมเทียมจากน้ำมันผสม

ขั้นตอนที่ 3 การศึกษาอายุการเก็บรักษาของครีมเทียมจากน้ำมันผสม

ขั้นตอนที่ 4 การวิเคราะห์ต้นทุน

ขั้นตอนที่ 5 การทดลองตลาดหรือการทดสอบการยอมรับของผู้บริโภค

โดยอาสาสมัครจะมีส่วนสำคัญในการทดสอบทางประสาทสัมผัส (ทดสอบชิม) ในขั้นตอนที่ 2 และ 3 ซึ่งในขั้นตอนที่ 2 นั้นอาสาสมัครจะได้รับตัวอย่างครีมเทียมในแต่ละสูตรเพื่อให้ประเมินความชอบและให้คะแนนแต่ละคุณลักษณะของแต่ละสูตร จนกระทั่งได้สูตรที่ดีที่สุดเพียงหนึ่งสูตรสำหรับครีมเทียมเหลวและหนึ่งสูตรสำหรับครีมเทียมผง ในขั้นตอนที่ 3 นั้น จะมีการทดสอบคุณภาพด้านกายภาพ เคมี และจุลินทรีย์ของผลิตภัณฑ์ควบคู่ไปกับการทดสอบชิม แต่เพื่อความปลอดภัยสำหรับอาสาสมัคร ทางคณะผู้วิจัยจะมีการประเมินการเสื่อมเสียของผลิตภัณฑ์จากทั้งทางกายภาพ เคมี และจุลินทรีย์ก่อน ว่าอยู่ในมาตรฐานความปลอดภัย จึงจะให้อาสาสมัครทดสอบชิม ถ้าคุณภาพทั้ง 3 ด้านหรือด้านใดด้านหนึ่ง ไม่ได้มาตรฐาน ทางทีมผู้วิจัยจะหยุดการทดสอบชิมทันที

5. กระบวนการวิจัย ระยะเวลาที่อาสาสมัครจะต้องปฏิบัติ และจำนวนอาสาสมัคร

กระบวนการวิจัย

งานวิจัยนี้ได้แบ่งออกเป็น 5 ขั้นตอน ดังนี้

ขั้นตอนที่ 1 การศึกษาคุณสมบัติของน้ำมันปาล์มโอเลอีนและน้ำมันรำข้าวที่นำมาผลิตครีมเทียม

โดยศึกษาคุณสมบัติทั้ง 3 ด้านคือ ด้านกายภาพได้แก่ สี ความหนืด ด้านเคมีได้แก่ ปริมาณ Tocopherol, ปริมาณ Tocotrienol, ปริมาณ γ-oryzanol, ปริมาณ Trans fat, Fatty acid profile และ Antioxidant activity (DPPH) และด้านจุลินทรีย์ได้แก่ จุลินทรีย์ทั้งหมด ยีสต์และรา และEscherichia coli

ขั้นตอนที่ 2 การพัฒนาสูตรสำหรับครีมเทียมจากน้ำมันผสม

ศึกษาจากสูตรมาตรฐานที่มีทั่วไปจากนั้นทำการพัฒนาสูตรโดยการปรับส่วนผสมเพื่อให้เหมาะสมกับน้ำมันตัวอย่างทั้งสองชนิด

ขั้นตอนที่ 3 การศึกษาอายุการเก็บรักษาของครีมเทียมจากน้ำมันผสม

ศึกษาอายุการเก็บรักษาของผลิตภัณฑ์สุดท้ายโดยจะเก็บรักษาครีมเทียมเหลวไว้ที่อุณหภูมิตู้เย็น (4-8°C) ระยะเวลา 14 วัน (ทำการทดสอบคุณภาพทางกายภาพ เคมี และจุลินทรีย์ ทุกๆ 1 วัน) และเก็บรักษาครีมเทียมผงไว้ที่อุณหภูมิห้อง (25-30°C) ระยะเวลา 12 เดือน (ทำการทดสอบคุณภาพทางกายภาพ เคมี และจุลินทรีย์ ทุกๆ 1 วัน)

ขั้นตอนที่ 4 การวิเคราะห์ต้นทุน

เพื่อให้ทราบถึงจุดคุ้มทุนหรือความเป็นไปได้ในการลงทุนและทราบจำนวนการผลิตในแต่ละครั้งว่าเหมาะสมหรือไม่

ขั้นตอนที่ 5 การทดลองตลาดหรือการทดสอบการยอมรับของผู้บริโภค

เพื่อให้ทราบการยอมรับของผู้บริโภคต่อผลิตภัณฑ์ ความต้องการของผู้บริโภคและความเป็นไปได้ในการจัดจำหน่ายสู่ตลาดจึงจำเป็นต้องศึกษาการยอมรับของผู้บริโภค

ระยะเวลาที่อาสาสมัครจะต้องปฏิบัติ 1 ปี

จำนวนอาสาสมัคร 50 คน สำหรับขั้นตอนที่ 2 และ3 และจำนวนอาสาสมัคร 400 คน สำหรับขั้นตอนที่ 5

6. ผลประโยชน์ที่คาดว่าจะเกิดขึ้นจากการทำวิจัย ระบุประโยชน์โดยตรงและ/หรือที่อาสาสมัครจะได้รับ ประโยชน์ต่อชุมชน ต่อสังคม และความรู้ด้านวิทยาศาสตร์

.....ความเป็นไปได้สำหรับการผลิตครีมเทียมเหลวและผงจากน้ำมันปาล์มผสมน้ำมันรำข้าว.....

7. ทางเลือกอื่นของการรักษา รวมทั้งประโยชน์ที่อาสาสมัครอาจได้รับ

.....ประโยชน์ที่อาสาสมัครอาจได้รับคือการทราบถึงกระบวนการทดสอบทางประสาทสัมผัสการรับรสและได้ประโยชน์จากสารอาหารที่มีอยู่ในผลิตภัณฑ์

8. ความเสี่ยงหรือความไม่สบายที่อาจจะเกิดขึ้นแก่อาสาสมัครจากการเข้าร่วมโครงการ

.....ไม่มีความเสี่ยงที่อาจจะเกิดขึ้น.....

และระบุว่าการเข้าร่วมในการวิจัยอาจมีความเสี่ยงที่ไม่ได้คาดการณ์ไว้เกิดขึ้น

.....ไม่มีความเสี่ยงที่ไม่ได้คาดการณ์ไว้.....

9. ค่าชดเชยการเสียเวลา/ค่าเดินทาง/ค่าตอบแทน หรืออื่นๆที่อาสาสมัครจะได้รับ (ถ้ามี)

.....มีการแจกของชำร่วยหรือของที่ระลึกเล็กๆ น้อยๆ เช่น ปากกา สมุด ดินสอ.....

10. ค่าใช้จ่ายที่อาสาสมัครเข้าร่วมโครงการ ต้องจ่ายเอง

.....ไม่มี.....

11. สถานการณ์ที่อาจเป็นไปได้และ/หรือเหตุผลซึ่งต้องยุติการเข้าร่วมในการวิจัยของอาสาสมัคร

.....ไม่มี.....

12. แผนการทำลายข้อมูลหรือสิ่งส่งตรวจจะทำลายทิ้งเมื่อสิ้นสุดการวิจัย

.....ไม่มี.....

13. การรักษาและ/หรือสิ่งชดเชย ที่อาสาสมัครจะได้รับ (โดยไม่คิดมูลค่า) ในกรณีที่เกิดอันตรายอันเนื่องมาจากการวิจัย.....ไม่มี.....

14. ข้อมูลส่วนตัวของอาสาสมัครถูกเก็บรักษาเป็นความลับ โดยจะไม่มีการเปิดเผยชื่อของอาสาสมัคร หากผลการวิจัยได้รับการตีพิมพ์เผยแพร่

15. การเข้าร่วมเป็นอาสาสมัครในการวิจัยเป็นความสมัครใจ และอาสาสมัครอาจปฏิเสธที่จะเข้าร่วมหรือสามารถถอนตัวออกจากการวิจัยได้ทุกขณะ โดยไม่มีความผิดหรือสูญเสียประโยชน์ ซึ่งอาสาสมัครพึงจะได้รับ

16. แผนการเก็บรักษาสิ่งส่งตรวจเพื่อวิจัยในอนาคต.....ไม่มี.....

17. หากท่านมีคำถามหรือมีความวิตกกังวลเกี่ยวกับการศึกษานี้ ท่านสามารถติดต่อบุคคลดังต่อไปนี้

ชื่อ ผศ. ดร. เหรียญทอง สิงห์จานุสงค์ ที่อยู่ที่สามารถติดต่อได้ ภาควิชาอุตสาหกรรมเกษตร คณะเกษตรศาสตร์ ทรัพยากรธรรมชาติและสิ่งแวดล้อม มหาวิทยาลัยนเรศวร

โทรศัพท์ (ในเวลาราชการ) 055-962742

โทรศัพท์ (นอกเวลาราชการ) 089-6441918

18. ท่านสามารถสอบถามถึงสิทธิของอาสาสมัคร/แจ้งเรื่องร้องเรียน ได้ที่

คณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัย นเรศวร กองบริหารการวิจัย ชั้น 2 อาคารมหาธรรมราชา ต.ท่าโพธิ์ อ.เมือง จ.พิษณุโลก 65000 เบอร์โทร 055-968642 โทรสาร. 055-968604

APPENDIX D INFORMED CONSENT FORM

"This participant information sheet is in Thai, Please contract the authors

For English version"

หนังสือแสดงความยินยอมการเข้าร่วมโครงการวิจัย

INFORMED CONSENT FORM

โครงการวิจัยเรื่อง อัตราส่วนที่เหมาะสมระหว่างไขมันแข็ง (ปาล์มโอเลอีน) และน้ำมันรำข้าวในกระบวนการผลิตครีมเทียมเหลว และครีมเทียมผง

ข้าพเจ้า (นาย,นาง,นางสาว).....นามสกุล.....อายุ.....ปี

บัตรประชาชน/ข้าราชการเลขที่.....อยู่บ้านเลขที่.....หมู่ที่.....ตำบล.....อำเภอ.....จังหวัด.....

เกี่ยวกับการเป็นอาสาสมัครในโครงการวิจัย.... อัตราส่วนที่เหมาะสมของน้ำมันปาล์มโอเลอีนและน้ำมันรำข้าวในกระบวนการผลิตครีมเทียมเหลว และครีมเทียมผง...ได้รับทราบถึงรายละเอียดของโครงการวิจัยเกี่ยวกับ

- วัตถุประสงค์และระยะเวลาที่ทำการวิจัย (ระบุ)...ทดสอบชิมเพื่อให้อาสาสมัครระบุคะแนนความชอบในแต่ละผลิตภัณฑ์โดยใช้เวลาในการทดสอบชิมแต่ละครั้งประมาณ 15 นาที

- ขั้นตอนและวิธีการปฏิบัติตัวที่ข้าพเจ้าต้องปฏิบัติ (ระบุ)...เตรียมทดสอบชิมผลิตภัณฑ์ครีมเทียมโดยใช้ชงผสมในกาแฟ และทำการตอบแบบสอบถามโดยการให้คะแนนความชอบตามใบประเมินที่แจกให้

- ผลประโยชน์ที่ข้าพเจ้าจะได้รับ (ระบุ)...ได้ทราบถึงกระบวนการทดสอบทางด้านประสาทสัมผัสและได้รับสารที่เป็นประโยชน์จากผลิตภัณฑ์

- ผลข้างเคียงหรืออันตรายที่อาจเกิดขึ้นจากการเข้าร่วมโครงการได้แก่.....ไม่มี..... และหากเกิดมีอาการข้างเคียงขึ้น ข้าพเจ้าจะรายงานให้ผู้วิจัยทราบทันที (ขอให้ผู้วิจัยระบุรายละเอียดตามความเหมาะสมให้สอดคล้องกับลักษณะโครงการ)

- ในกรณีที่โครงการวิจัยนี้เกี่ยวกับการรักษาพยาบาลขอให้คงข้อความนี้ไว้

"หากข้าพเจ้าถอนตัวจากการศึกษาครั้งนี้ ข้าพเจ้าจะไม่เสียสิทธิ์ใดๆ ในการรับการรักษาพยาบาลที่จะเกิดขึ้นตามมาในโอกาสต่อไป ทั้งในปัจจุบันและอนาคต ณ สถานพยาบาลแห่งนี้หรือสถานพยาบาลอื่น"

- ข้าพเจ้าสามารถถอนตัวจากการศึกษานี้เมื่อใดก็ได้ถ้าข้าพเจ้าปรารถนา โดยไม่มีการเสียสิทธิ์ใดๆ ทั้งสิ้น

- ผู้วิจัยและ/หรือผู้ให้ทุนสนับสนุนการวิจัยขอให้คำรับรองว่าจะเก็บข้อมูลเกี่ยวกับข้าพเจ้าเป็นความลับและจะเปิดเผยเฉพาะในรูปที่เป็นการสรุปการวิจัย โดยไม่ระบุตัวบุคคลผู้เป็นเจ้าของข้อมูล และหากเกิดอันตรายหรือความเสียหายอันเป็นผลจากการวิจัยต่อข้าพเจ้า ผู้วิจัยและ/หรือผู้ให้ทุนสนับสนุนการวิจัยจะจัดการรักษาพยาบาลให้จนกลับคืนสภาพเดิม และจะเป็นผู้ออกค่าใช้จ่ายทั้งหมดในการรักษาพยาบาลรวมทั้งชดใช้ค่าเสียหายอื่นถ้าหากมี

ข้าพเจ้าได้อ่านและเข้าใจคำอธิบายข้างต้นแล้ว จึงได้ลงนามยินยอมเป็นอาสาสมัครของโครงการวิจัยดังกล่าว

ลายมือชื่ออาสาสมัคร.....

(.....)

ลายมือชื่อผู้ปกครอง.....

(.....)

ลายมือชื่อผู้ให้ข้อมูล.....

(.....)

พยาน.....(ไม่ใช่ผู้อธิบาย)

(.....)

วันที่.....เดือน.....พ.ศ.....

หมายเหตุ :

1) ในกรณีที่อาสาสมัครมีอายุต่ำกว่า 20 ปีบริบูรณ์ และสามารถตัดสินใจเองได้ ให้ลงลายมือชื่อทั้งอาสาสมัคร (เด็ก) และผู้ปกครองด้วย
2) พยานต้องไม่ใช่ผู้วิจัย หรือผู้ร่วมวิจัย และผู้มีส่วนได้ส่วนเสียกับโครงการวิจัย
3) ผู้ให้ข้อมูล/คำอธิบาย ต้องไม่เป็นแพทย์ที่ทำโครงการวิจัยนี้ด้วยตนเอง เพื่อป้องกันการเข้าร่วมโครงการด้วยความเกรงใจ
4) ในกรณีที่อาสาสมัครไม่สามารถ อ่านหนังสือ/ลงลายมือชื่อได้ ให้ใช้การประทับลายมือแทนดังนี้ :

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในแบบคำยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดี ข้าพเจ้าจึงประทับตราลายนิ้วมือขวาของข้าพเจ้าในแบบคำยินยอมนี้ด้วยความเต็มใจ

ประทับลายนิ้วมือขวา

ลายมือชื่อผู้อธิบาย.....

(.....)

พยาน.....(ไม่ใช่ผู้อธิบาย)

(.....)

วันที่.....เดือน.....พ.ศ.....

หมายเหตุ: ขอให้ผู้วิจัยระบุรายละเอียดตามความเหมาะสมให้สอดคล้องกับลักษณะโครงการ

APPENDIX E ASSESSMENT FORM

ผลิตภัณฑ์....ครีมเทียมเหลว.... ครั้งที่.....

ชื่อ สกุลผู้ทดสอบ..... วันที่ทดสอบ.....

กรุณาชิมผลิตภัณฑ์ทั้ง 3 ตัวอย่างตามลำดับที่นำเสนอ แล้วประเมินความชอบต่อผลิตภัณฑ์โดยให้คะแนนที่ตรงกับความคิดเห็นของท่านในคุณลักษณะดังต่อไปนี้

(กรุณาดื่มน้ำระหว่างตัวอย่าง)

คะแนนความชอบ

1 = ไม่ชอบมากที่สุด	4 = ไม่ชอบเล็กน้อย	7 = ชอบปานกลาง
2 = ไม่ชอบมาก	5 = เฉยๆ	8 = ชอบมาก
3 = ไม่ชอบปานกลาง	6 = ชอบเล็กน้อย	9 = ชอบมากที่สุด

	ตัวอย่าง	.....	.....	.....
สีของครีมเทียมเหลว		.....	.....	.....
ความละเอียดของครีมเทียมเหลว		.....	.....	.....
กลิ่นครีมเทียมเหลว		.....	.....	.....
ความสามารถในการละลายของครีมเทียมเหลว		.....	.....	.....
สีของกาแฟ		.....	.....	.....
การเกิดฝ้า		.....	.....	.....
รสชาติของกาแฟ		.....	.....	.....
เนื้อสัมผัสของกาแฟ		.....	.....	.....
คุณภาพโดยรวม		.....	.....	.....

ข้อเสนอแนะ_____

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-----ขอบคุณที่สละเวลาในการทดสอบ-----

ผลิตภัณฑ์....ครีมเทียมผง.... ครั้งที่.....

ชื่อ สกุลผู้ทดสอบ..... วันที่ทดสอบ.....

กรุณาชิมผลิตภัณฑ์ทั้ง 3 ตัวอย่างตามลำดับที่นำเสนอ แล้วประเมินความชอบต่อผลิตภัณฑ์โดยให้คะแนนที่ตรงกับความคิดเห็นของท่านในคุณลักษณะดังต่อไปนี้

(กรุณาดื่มน้ำระหว่างตัวอย่าง)

คะแนนความชอบ

1 = ไม่ชอบมากที่สุด	4 = ไม่ชอบเล็กน้อย	7 = ชอบปานกลาง
2 = ไม่ชอบมาก	5 = เฉยๆ	8 = ชอบมาก
3 = ไม่ชอบปานกลาง	6 = ชอบเล็กน้อย	9 = ชอบมากที่สุด

ตัวอย่าง	.....	.....	.....
สีของครีมเทียมผง	.....	.....	.....
ความละเอียดของครีมเทียมผง	.....	.....	.....
กลิ่นครีมเทียมผง	.....	.....	.....
ความสามารถในการละลายของครีมเทียมผง	.....	.....	.....
ความสามารถในการกระจายตัวของครีมเทียมผง	.....	.....	.....
สีของกาแฟ	.....	.....	.....
การเกิดฝ้า	.....	.....	.....
รสชาติของกาแฟ	.....	.....	.....
เนื้อสัมผัสของกาแฟ	.....	.....	.....
คุณภาพโดยรวม	.....	.....	.....

ข้อเสนอแนะ_____

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-----ขอบคุณที่สละเวลาในการทดสอบ-----

แบบสอบถามเพื่อการพัฒนาผลิตภัณฑ์

คำชี้แจง : กรุณาทำเครื่องหมาย ✔ ลงในกรอบสี่เหลี่ยมที่ท่านเห็นว่าเหมาะสมที่สุด

หมายเหตุ การตอบแบบสอบถามนี้เป็นการให้ข้อมูลที่เป็นประโยชน์ต่อการพัฒนาผลิตภัณฑ์ให้ตรงกับความต้องการของผู้บริโภค มีทั้งหมด 4 ตอน กรุณาทำตามคำอธิบายในแต่ละตอน

ตอนที่ 1 ข้อมูลทั่วไปเกี่ยวกับผู้บริโภค

1. เพศ

☐ ชาย ☐ หญิง

2. อายุ

☐ ต่ำกว่า 20 ปี ☐ 20 – 25 ปี ☐ 26 – 35 ปี

☐ 36 – 45 ปี ☐ 46 – 55 ปี ☐ 56 ปีขึ้นไป

3. อาชีพ

☐ นักเรียน ☐ นิสิต / นักศึกษา ☐ ข้าราชการ

☐ พนักงานเอกชน ☐ พนักงานรัฐวิสาหกิจ ☐ เกษตรกร

☐ รับจ้าง ☐ แม่บ้าน ☐ อื่น ๆ โปรดระบุ.....

4.รายได้ต่อเดือนของครอบครัว

☐ น้อยกว่า 6,000 บาท ☐ 6,001 – 9,000 บาท

☐ 9,001 – 12,000 บาท ☐ 12,001 – 15,000 บาท

☐ 15,001 – 18,000 บาท ☐ อื่น ๆ โปรดระบุ.....

5. สภาพการอยู่อาศัย

☐ คนเดียว

☐ ครอบครัว จำนวนสมาชิก.....คน (รวมท่านด้วย)

☐ อยู่ร่วมกับผู้อื่น จำนวนสมาชิก.....คน (รวมท่านด้วย)

6. การศึกษา

☐ ประถมศึกษา ☐ อนุปริญา / ปวส. ☐ มัธยมต้น (ม.3)

☐ ปริญญาตรี ☐ มัธยมปลาย (ม.6) / ปวช. ☐ สูงกว่าปริญญาตรี

ตอนที่ 2 ข้อมูลเกี่ยวกับพฤติกรรมการบริโภคครีมเทียม

7. ท่าน**เคย**รับประทานครีมเทียมหรือไม่

☐ เคย (กรุณาทำต่อไป) ☐ ไม่เคย (กรุณาข้ามไปทำตอนที่ 3 - 4)

8. ท่าน**ชอบ**รับประทานครีมเทียมหรือไม่

☐ ชอบ (กรุณาทำข้อ 9 ต่อไป) ☐ ไม่ชอบ (กรุณาทำข้อ 10 ต่อไป)

9. เหตุผลที่ท่าน**ชอบ**รับประทานครีมเทียม

☐ รสชาติ ☐ เนื้อสัมผัส ☐ ทำให้กาแฟมีสีและรสชาติกลมกล่อมขึ้น

☐ กลิ่น ☐ คุณค่าทางโภชนาการ ☐ อื่น ๆ โปรดระบุ .....

(กรุณาทำข้อ 11 ต่อไป)

10. เหตุผลที่ท่าน**ไม่ชอบ**รับประทานครีมเทียม

☐ รสชาติ ☐ ราคาแพง ☐ กลิ่น

☐ เนื้อสัมผัส ☐ อื่น ๆ โปรดระบุ .....

(กรุณาข้ามไปทำตอนที่ 3 - 4)

11. ความถี่ในการรับประทานครีมเทียม

☐ ประจำ ระบุ

☐ วันละ 1 ครั้ง ☐ วันละ 2 ครั้ง

☐ วันละ 3 ครั้ง ☐ มากกว่าวันละ 3 ครั้ง

☐ ครั้งคราว ระบุ

☐ สัปดาห์ละ 1 ครั้ง ☐ เดือนละ 1 ครั้ง

☐ เดือนละ 2-3 ครั้ง

☐ นานๆ ครั้ง

12. ปกติท่านนำมาใช้ร่วมกับอาหารชนิดใด

☐ ชา ☐ กาแฟ

☐ โกโก้ ☐ อื่นๆ โปรดระบุ.....

13. สถานที่ใดที่ท่านมักจะซื้อครีมเทียมมาเพื่อรับประทาน

☐ ห้างสรรพสินค้า (Big C, Lotus, Makro) ☐ ร้านสะดวกซื้อ (7-11, 108 Shop)

☐ ร้านขายของชำ ☐ อื่นๆ โปรดระบุ.....

14. ปกติท่านซื้อผลิตภัณฑ์ครีมเทียมขนาดบรรจุเท่าไรมารับประทาน

☐ 1 กรัม ☐ 5 กรัม

☐ 10 กรัม ☐ 15 กรัม

☐ 20 กิโลกรัม ☐ อื่นๆโปรดระบุ.....

ตอนที่ 3 ข้อมูลเกี่ยวกับผลิตภัณฑ์ที่พัฒนา (กรุณาทำแบบสอบถามให้ครบทุกข้อตามคำอธิบาย)

15. ท่านรู้จักครีมเทียมหรือทราบข้อมูลที่เกี่ยวข้องกับครีมเทียมบ้างหรือไม่

☐ เคย (กรุณาทำข้อต่อไป) ☐ ไม่เคย (กรุณาข้ามไปทำข้อ 17 ต่อไป)

16. ท่านเคยรับประทานครีมเทียมหรือไม่

☐ เคย (กรุณาทำข้อต่อไป) ☐ ไม่เคย (กรุณาทำข้อต่อไป)

17. หากมีการผลิต**ครีมเทียมผงและครีมเทียมเหลว**ท่านสนใจหรือไม่

☐ สนใจ (กรุณาทำข้อ 18 ต่อไป)

☐ ไม่สนใจ (กรุณาทำข้อ 19 ต่อไป)

18. เหตุผลที่ท่าน<u>**สนใจ**</u>หากมีการผลิต**ครีมเทียมผงและครีมเทียมเหลว** เพราะ

☐ ความแปลกใหม่ ☐ คุณค่าทางโภชนาการ ☐ ราคา

☐ รับประทานเป็นประจำอยู่แล้ว ☐ อื่นๆ โปรดระบุ .....

19. เหตุผลที่ท่าน<u>**ไม่สนใจ**</u>หากมีการผลิต**ครีมเทียมผง**และ**ครีมเทียมเหลว**เพราะ

☐ ไม่กล้ารับประทาน เพราะ.....

☐ ไม่ดื่มชา กาแฟ หรือผลิตภัณฑ์ที่ต้องมีการเติมครีมเทียมผงและครีมเทียมเหลว

☐ อื่นๆ โปรดระบุ .....

ตอนที่ 4 ข้อมูลของลักษณะครีมเทียมที่ผู้บริโภคต้องการ (กรุณาทำแบบสอบถามให้ครบทุกข้อตามคำอธิบาย)

20. ลักษณะที่สำคัญที่สุดที่ท่านต้องการในครีมเทียมคือ

☐ รสชาติ ☐ คุณค่าทางโภชนาการ ☐ กลิ่น

☐ สี / ลักษณะปรากฏ ☐ เนื้อสัมผัส ☐ อื่น ๆ โปรดระบุ .....

21. ลักษณะภาชนะบรรจุครีมเทียมที่ท่านชอบสำหรับ**ครีมเทียมเหลว**

☐ บรรจุถุงพลาสติก ☐ บรรจุถ้วยพลาสติก ☐ บรรจุขวดแก้ว

☐ บรรจุอะลูมิเนียมฟอยด์ ☐ อื่น ๆ โปรดระบุ.....

22. ลักษณะภาชนะบรรจุครีมเทียมที่ท่านชอบสำหรับ**ครีมเทียมผง**

☐ บรรจุถุงพลาสติก ☐ บรรจุถ้วยพลาสติก ☐ บรรจุขวดแก้ว

☐ บรรจุอะลูมิเนียมฟอยด์ ☐ อื่น ๆ โปรดระบุ.....

23. น้ำหนักต่อหนึ่งหน่วยบรรจุที่ท่านคิดว่าเหมาะสมสำหรับ**ครีมเทียมเหลว**

☐ 15 มิลลิลิตร ต่อ ถ้วยพลาสติก ☐ 20 มิลลิลิตร ต่อ ถ้วยพลาสติก

☐ 100 มิลลิลิตร ต่อ ขวดพลาสติก ☐ 200 มิลลิลิตร ต่อ ขวดพลาสติก

☐ 100 มิลลิลิตร ต่อ ขวดแก้ว ☐ 200 มิลลิลิตร ต่อ ขวดแก้ว

☐ อื่น ๆ โปรดระบุ.....

24. น้ำหนักต่อหนึ่งหน่วยบรรจุที่ท่านคิดว่าเหมาะสมสำหรับ**ครีมเทียมผง**

☐ 3 กรัม ต่อ 50 ซองพลาสติก ☐ 3 กรัม ต่อ 100 ซองพลาสติก

☐ 75 กรัม ต่อ ถุงอะลูมิเนียมฟอยด์ ☐ 100 กรัมต่อ ถุงอะลูมิเนียมฟอยด์

☐ 200 กรัม ต่อ ถุงอะลูมิเนียมฟอยด์ ☐ 400 กรัม ต่อ กระปุกพลาสติก

☐ อื่น ๆ โปรดระบุ.....

25. หากมีการกำหนดราคาขายต่อหนึ่งหน่วยบรรจุราคาเท่าไรท่านคิดว่าเหมาะสมและเต็มใจที่จะซื้อสำหรับ**ครีมเทียมเหลว** (ดังน้ำหนักต่อหนึ่งหน่วยบรรจุในข้อ 23)

☐ 1 - 5 บาท ☐ 6 - 10 บาท ☐ 11 - 30 บาท ☐ 31 - 60 บาท

☐ 61 - 90 บาท ☐ 91 - 120 บาท ☐ อื่นๆ โปรดระบุ .....

26. หากมีการกำหนดราคาขายต่อหนึ่งหน่วยบรรจุราคาเท่าไรท่านคิดว่าเหมาะสมและเต็มใจที่จะซื้อสำหรับ**ครีมเทียมผง** (ดังน้ำหนักต่อหนึ่งหน่วยบรรจุในข้อ 24)

☐ 30 - 40 บาท ☐ 60 - 70 บาท ☐ 15 - 20 บาท

☐ 25 - 35 บาท ☐ 70 – 80 บาท ☐ อื่นๆ โปรดระบุ .....

27. <u>ปัจจัยที่สำคัญที่สุด</u> ที่มีอิทธิพลต่อการตัดสินใจซื้อครีมเทียม

☐ ราคา ☐ คุณค่าทางโภชนาการ

☐ ชนิดของภาชนะบรรจุและวิธีการบรรจุ ☐ ปริมาณบรรจุต่อหนึ่งหน่วยบรรจุ

☐ สถานที่จำหน่าย ☐ ความสะดวกสบาย

☐ อื่น ๆ โปรดระบุ.....

ความคิดเห็น / ข้อเสนอแนะเพิ่มเติม

.....

.....

ขอขอบพระคุณสำหรับการสละเวลากรอกแบบสอบถาม

APPENDIX F PHYSICAL DETERMINATION

Color value (Hunter lab L*, a*, b*)

Color of the samples was measured by colorimeter according to the method Li et al. (2014). The sample was poured in the plate 30 g and measured by Hunter Lab Color flex model 4510 from USA. The color result was expressed in CIE-LAB (L*, a* and b*) which showed the parameters, including L* (lightness); a* (red green axis), and b* (yellow blue axis). The colorimeter was calibrated using a white and black standard plate before use.

Absorbance (UV-VIS spectrophotometry)

Absorbance of the samples was measured by UV-VIS spectrophotometry. 5 mL of sample was transferred to a cuvette (3 mL). Absorbance of HPKO and CRBO was detected at the visible wavelength of 380-750 nm.

Viscosity (Brookfield)

Viscosity of the samples was measured by Brookfield. Added 100 mL of sample in beaker. Probe No. 5 was used to detect the viscosity and collect data.

Solubility (Al – khatani and Hassan, 1990)

Weigh the sample 10 g, dissolve in distill water (room temperature) 250 ml. in 500 ml beaker. Mixed well by magnetic stirrer at level 5. Collect data after completely dissolve.

Bulk density (Nezbed, 1973)

Bulk density was weighed of product one unit capacity (g) per cubic centimeter (g/cm^3). Determine by weighting the powder that know weigh transfer to cylinder 10 ml. Shack until constant and record. This experimental was 3 replicates. Calculate following

$$\text{Bulk density} = \text{weight/capacity}$$

APPENDIX G CHEMICAL DETERMINATION

Fat content (AOAC, 1999)

Dried sample and weighed 2 g of sample into a thimble. Transferred to soxhlet extractor, extract sample by using petroleum ether 130 ml for 16 h. Evaporated petroleum ether by using hot air oven at 60°C for 30 min, left in desiccator until cool, replete until weigh constant. Calculated % fat following:

$$\%Fat = (\text{weigh of oil} \times 100)/\text{weigh of sample}$$

Moisture content (Kha, Nguyen and Roach, 2010)

The moisture content of the sample was analyzed according to the method of Kha, Nguyen and Roach (2010). Two g of the sample were dried in a hot air oven (Lab Tech model LDO-150F, South Korea) at the temperature of 105°C until a constant weight was obtained.

Water activity (Kha, Nguyen and Roach, 2010)

One g of the samples were determined for water activity by a water activity meter (AquaLab model Pre series 4TE, USA) at the temperature of 25 °C.

Acid value (AOCS, 1995)

Reagent

1. N/50 KOH ethanol (0.02 N KOH in ethyl alcohol) prepared by weighed 5.6g of KOH dissolve in water adjust to 100 mL 20 mL of solution adjust to 1,000 mL by ethanol.

2. n-Hexane

3. 1% phenolphthalein in ethanol (Indicator) prepared by 1g of phenolphthalein dissolve in 100 mL of ethanol.

Procedure

1. 1g of sample put in Erlenmeyer flask

2. Added 10 time of n-Hexane with sample after that 1-2 drops of phenolphthalein was added.

3. Titrate solution with N/50 KOH ethanol until end point

4. Prepared Blank by using C-M solution

Calculated:

$$\text{Acid Value} = 56.1 \times \text{N of KOH } (Vs - Vb)/Ws$$

Where N of KOH = normality of KOH

Vs = amount of KOH titrate with sample

Vb = amount of KOH titrate with blank

Ws = weigh of sample

Total acidity (as lactic acid) (Tseng and Zhao, 2013)

Total acidity was analyzed according to the method of Tseng and Zhao (2013). The sample was weighed 2 g into the Erlenmeyer flask. Distill water 50 mL was added in the flask and 1 mL of 1% phenolphthalein solution was added. The solution was swirled until well mixing and titrated with 0.1 N of sodium hydroxide until appearing pink color. Volume of sodium hydroxide solution was recorded. Total acidity was calculated and reported as acetic acid according to the following equation

$$\text{Total acidity} = \frac{(\text{mL NaOH} \times \text{NaOH normality} \times 60.5 \times 100)}{(\text{weight of sample (g)} * 1000)}$$

Titratable acidity is expressed as % lactic acid (MW = 60.5)

Antioxidant activity (DPPH) (Anagnostopoulou et al, 2006)

Antioxidant activities were determined in accordance with the method of Anagnostopoulou, et al. (2006) with slight modifications. These dilutions of each aqueous extract, 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 blank for

30 min in the dark at room temperature. The percentage of scavenging activity of DPPH was calculated according to the following equation. The linear curve were plotted between percentage of radical scavenging and sample concentration.

$$\text{Radical scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}]*100$$

($A_{control}$ = absorbance without extract; A_{sample} = absorbance with extract)

Free fatty acid value (FFA) (Wrolstad et al, 2005)

FFA is defined as the percentage by weight of free acid groups in the oil. The method was analyzed according to the method of Wrolstad et al. (2005). The oil extract was weighed 0.2 g into the Erlenmeyer flask. Preheating to 60°C ethanol 50 mL was added in the flask and 1 mL of 1% phenolphthalein solution was added. The solution was swirled until well mixing and titrated with 0.1 N sodium hydroxide until appearing a pink color. Volume of sodium hydroxide solution was recorded. FFA was calculated and reported as oleic acid according to the following equation.

$$\text{FFA} = (\text{mL NaOH} \times \text{NaOH normality} \times 28.2)/\text{Weight of sample (g)}$$

as % oleic acid

Peroxide value (Wrolstad, et al. 2005)

The peroxide value of lipid extract is defined as the quantity of peroxide oxygen present in the sample. The PV was analyzed according to the method of Wrolstad et al. (2005). Five g lipid extract was poured into a 250 Ehrlenmeyer 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 is dissolved. This step was done in a fume hood to avoid in halation of the vapors carcinogen from chloroform. Saturated potassium iodide solution 0.5 mL was added in flask and thoroughly mixed. The solution was maintained 1 min with occasional shaking and then 30 mL of distilled water was added. 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 disappeared.

A blank was prepared using the reaction solvent without lipid extract. Peroxide value was calculated according to the following equation.

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

Where S is the volume (mL) of sodium thiosulfate used to titrate the sample, B is volume (mL) of sodium thiosulfate used for the blank, N is the normality of the standardized sodium thiosulfate solution and W is the weight of the sample (g).

Fatty acid profile, γ-Oryzanol, α-tocopherol, Trans fat

These were analyzed by Institute of Food Research and Product Development. Kasetsart University, Bangkok, Thailand. The method as following:

Fatty acid profile: In house method based on Compendium of Methods for Food Analysis. Thailand, 2003.

γ-Oryzanol: In house method based on ASEAN Food Journal, 2008, 15 (1), 89-96.

α-Tocopherol: In house method based on Journal of Chromatography A, 1991, 825, 127-133.

Trans fat: In house method based on Compendium of Methods for Food Analysis. Thailand, 2003.

APPENDIX H MICROBIOLOGICAL DETERMINATION

Total plate count (Maturin and Peeler, 2001)

A. Equipment and materials

1. Work area, level table with ample surface in room that is clean, well-lighted (100 foot-candles at working surface) and well-ventilated, and reasonably free of dust and drafts. The microbial density of air in working area, measured in fallout pour plates taken during plating, should not exceed 15 colonies/plate during 15 min exposure.

2. Storage space, free of dust and insects and adequate for protection of equipment and supplies

3. Petri dishes, glass or plastic (at least 15 × 90 mm)

4. Pipets with pipet aids (no mouth pipetting) or pipettors, 1, 5, and 10 ml, graduated in 0.1 ml units

5. Dilution bottles, 6 oz (160 ml), borosilicate-resistant glass, with rubber stoppers or plastic screw caps

6. Pipet and petri dish containers, adequate for protection

7. Circulating water bath, for tempering agar, thermostatically controlled to 45 ± 1°C

8. Incubator, 35 ± 1°C; milk, 32 ± 1°C

9. Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate

10. Tally register

11. Dilution blanks, 90±1 ml Butterfield's phosphate-buffered dilution water (R11); milk, 99±2 ml

12. Plate count agar (standard methods) (M124)

13. Refrigerator, to cool and maintain samples at 0-5°C; milk, 0-4.4°C

14. Freezer, to maintain frozen samples from -15 to -20°C

15. Thermometers (mercury) appropriate range; accuracy checked with a thermometer certified by the National Institute of Standards and Technology (NIST)

B. Procedure for analysis of frozen, chilled, precooked, or prepared foods

Using separate sterile pipets, prepare decimal dilutions of 10-2, 10-3, 10-4, and others as appropriate, of food homogenate by transferring 10 ml of previous dilution to 90 ml of diluent. Avoid sampling foam. Shake all dilutions 25 times in 30 cm (1 ft) arc within 7 s. Pipet 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes. Reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into petri dish. Add 12-15 ml plate count agar (cooled to 45±1°C) to each plate within 15 min of original dilution. For milk samples, pour an agar control, pour a dilution water control and pipet water for a pipet control. Add agar to the latter two for each series of samples. Add agar immediately to petri dishes when sample diluent contains hygroscopic materials, e.g., flour and starch. Pour agar and dilution water control plates for each series of samples. Immediately mix sample dilutions and agar medium thoroughly and uniformly by alternate rotation and back-and-forth motion of plates on flat level surface. Let agar solidify. Invert solidified petri dishes, and incubate promptly for 48±2 h at 35°C. Do not stack plates when pouring agar or when agar is solidifying.

C. Guidelines for calculating and reporting APCs in uncommon cases

Official Methods of Analysis does not provide guidelines for counting and reporting plate counts, whereas Standard Methods for the Examination of Dairy Products, 16th ed. presents detailed guidelines; for uniformity, therefore, use APHA guidelines as modified. Report all aerobic plate counts computed from duplicate plates. For milk samples, report all aerobic plate counts computed from duplicate plates containing less than 25 colonies as less than 25 estimated count. Report all aerobic plate counts computed from duplicate plates containing more than 250 colonies as estimated counts. Counts outside the normal 25-250 range may give erroneous indications of the actual bacterial composition of the sample. Dilution factors may exaggerate low counts (less than 25), and crowded plates (greater than 250) may be difficult to count or may inhibit the growth of some bacteria, resulting in a low count. Report counts less than 25 or more than 250 colonies as estimated aerobic plate counts (EAPC). Use the following guide:

1. Normal plates (25-250). Select spreader-free plate(s). Count all colony forming units (CFU), including those of pinpoint size, on selected plate(s). Record dilution(s) used and total number of colonies counted.

2. Plates with more than 250 colonies. When number of CFU per plate exceeds 250, for all dilutions, record the counts as too numerous to count (TNTC) for all but the plate closest to 250, and count CFU in those portions of plate that are representative of colony distribution. Mark calculated APC with EAPC to denote that it was estimated from counts outside 25-250 per plate range.

3. Spreaders. Spreading colonies are usually of 3 distinct types: 1) a chain of colonies, not too distinctly separated, that appears to be caused by disintegration of a bacterial clump; 2) one that develops in film of water between agar and bottom of dish; and 3) one that forms in film of water at edge or on surface of agar. If plates prepared from sample have excessive spreader growth so that (a) area covered by spreaders, including total area of repressed growth, exceeds 50% of plate area, or (b) area of repressed growth exceeds 25% of plate area, report plates as spreaders. When it is necessary to count plates containing spreaders not eliminated by (a) or (b) above, count each of the 3 distinct spreader types as one source. For the first type, if only one chain exists, count it as a single colony. If one or more chains appear to originate from separate sources, count each source as one colony. Do not count each individual growth in such chains as a separate colony. Types 2 and 3 usually result in distinct colonies and are counted as such. Combine the spreader count and the colony count to compute the APC.

4. Plates with no CFU. When plates from all dilutions have no colonies, report APC as less than 1 times the corresponding lowest dilution used. Mark calculated APC with asterisk to denote that it was estimated from counts outside the 25-250 per plate range. When plate(s) from a sample are known to be contaminated or otherwise unsatisfactory, record the result(s) as laboratory accident (LA).

Yeast and mold (Tournas, et al., 2001)

Enumeration of Yeasts and Molds in Food—Dilution Plating Technique

A. Equipment and materials

1. Basic equipment (and appropriate techniques) for preparation of sample homogenate
2. Equipment for plating samples
3. Incubator, 25°C
4. Arnold steam chest
5. pH meter
6. Water bath, 45 ± 1° C

B. Media and reagents

Media

1. Dichloran rose□engal chloramphenicol (DRBC) agar (M183)
2. Dichloran 18% glycerol (DG18) agar (M184)
3. Plate count agar (PCA), standard methods (M124); add 100 mg chloramphenicol/liter when this medium is used for yeast and mold enumeration. This medium is not efficient when "spreader" molds are present.
4. Malt agar (MA)(M185)
5. Malt extract agar (Yeasts and Molds) (MEAYM)(M182)
6. Potato dextrose agar (PDA), dehydrated; commercially available (M127)

Antibiotic solutions

Antibiotics are added to mycological media to inhibit bacterial growth. Chloramphenicol is the antibiotic of choice, because it is stable under autoclave conditions. Therefore, media preparation is easier and faster due to the elimination of the filtration step. The recommended concentration of this antibiotic is 100 mg/liter medium. If bacterial overgrowth is apparent, prepare media by adding 50 mg/liter chloramphenicol before autoclaving and 50 mg/liter filter-sterilized chlortetracycline when the media have been tempered, right before pouring plates.

Prepare stock solution by dissolving 0.1 g chloramphenicol in 40 ml distilled water; add this solution to 960 ml medium mixture before autoclaving. When both chloramphenicol and chlortetracycline are used, add 20 ml of the above

chloramphenicol stock solution to 970 ml medium before autoclaving. Then, prepare chlortetracycline stock solution by dissolving 0.5 g antibiotic in 100 ml distilled water and filter sterilize. Use 10 ml of this solution for each 990 ml of autoclaved and tempered medium. Refrigerate in the dark and re-use remaining stock solutions for up to a month. Stock solutions should be brought to room temperature before adding to tempered medium.

C. Procedures

Sample preparation

Analyze 25-50 g from each subsample; generally, larger sample sizes increase reproducibility and lower variance compared with small samples. Test individual subsamples or composite according to respective Compliance Program for the food under analysis. Add appropriate amount of 0.1% peptone water to the weighed sample to achieve 10-1 dilution, then homogenize in a stomacher for 2 min. Alternatively, blending for 30-60 sec can be used but is less effective. Make appropriate 1:10 (1+9) dilutions in 0.1% peptone water. Dilutions of 10-6 should suffice.

Plating and incubation of sample

Spread-plate method. Aseptically pipet 0.1 ml of each dilution on pre-poured, solidified DRBC agar plates and spread inoculum with a sterile, bent glass rod. DG18 is preferred when the water activity of the analyzed sample is less than 0.95. Plate each dilution in triplicate.

Pour-plate method. Use sterile cotton-plugged pipet to place 1.0 ml portions of sample dilution into prelabeled 15 × 100 mm Petri plates (plastic or glass), and immediately add 20-25 ml tempered DG18 agar. Mix contents by gently swirling plates clockwise, then counterclockwise, taking care to avoid spillage on dish lid. After adding sample dilution, add agar within 1-2 min; otherwise, dilution may begin to adhere to dish bottom (especially if sample is high in starch content and dishes are plastic) and may not mix uniformly. Plate each dilution in triplicate.

From preparation of first sample dilution to pouring or surface-plating of final plate, no more than 20 min (preferably 10 min) should elapse. **Note**: Spread plating of diluted sample is considered better than the pour plate method. When the pour plate technique is used, fungal colonies on the surface grow faster and often obscure those underneath the surface, resulting in less accurate enumeration. Surface plating gives a

more uniform growth and makes colony isolation easier. DRBC agar should be used for spread plates only.

Incubate plates in the dark at 25°C. Do not stack plates higher than 3 and do not invert. **Note**: Let plates remain undisturbed until counting.

Counting of plates

Count plates after 5 days of incubation. If there is no growth at 5 days, re-incubate for another 48 h. Do not count colonies before the end of the incubation period because handling of plates could result in secondary growth from dislodged spores, making final counts invalid. Count plates containing 10-150 colonies. If mainly yeasts are present, plates with 150 colonies are usually countable. However, if substantial amounts of mold are present, depending on the type of mold, the upper countable limit may have to be lowered at the discretion of the analyst. Report results in colony forming units (CFU)/g or CFU/ml based on average count of triplicate set. Round off counts to two significant figures. If third digit is 6 or above, round off to digit above (e.g., 456 = 460); if 4 or below, round off to digit below (e.g., 454 = 450). If third digit is 5, round off to digit below if first 2 digits are an even number (e.g., 445 = 440); round off to digit above if first 2 digits are an odd number (e.g., 455 = 460). When plates from all dilutions have no colonies, report mold and yeast counts (MYC) as less than 1 times the lowest dilution used.

Isolate individual colonies on PDA or MA, if further analysis and species identification is necessary.

Coliform and *Escherichia coli* (Feng, Weagent and Grant, 2002)

Conventional Method for coliforms, fecal coliforms and *E. coli*

A. Equipment and materials

1. Covered water bath, with circulating system to maintain temperature of 45.5±0.2°C.

Note: The temperature for water baths for the shellfish program is 44.5°C±0.2°C. Water level should be above the medium in immersed tubes.

2. Immersion-type thermometer, 1-55°C, about 55 cm long, with 0.1°C subdivisions, certified by National Institute of Standards and Technology (NIST), or equivalent

3. Incubator, 35±1.0°C

Note: The incubator temp for the shellfish program is 35°C±0.5°C

4. Balance with capacity of ≥2 kg and sensitivity of 0.1 g

5. Blender and blender jar

6. Sterile graduated pipets, 1.0 and 10.0 mL

7. Sterile utensils for sample handling

8. Dilution bottles made of borosilicate glass, with polyethylene screw caps equipped with Teflon liners. Commercially prepared dilution bottles containing sterile Butterfield's phosphate buffer can also be used.

9. Quebec colony counter, or equivalent, with magnifying lens

10. Longwave UV light [~365 nm], not to exceed 6 W.

11. pH meter

B. Media and Reagents

1. Brilliant green lactose bile (BGLB) broth, 2% (M25)

2. Lauryl tryptose (LST) broth (M76)

3. Lactose Broth (M74)

4. EC broth (M49)

5. Levine's eosin-methylene blue (L-EMB) agar (M80)

6. Tryptone (tryptophane) broth (M164)

7. MR-VP broth (M104)

8. Koser's citrate broth (M72)

9. Plate count agar (PCA) (standard methods) (M124)

10. Butterfield's phosphate-buffered water (R11) or equivalent diluent

(**Note**: This same formulation is referred to as Buffered Dilution Water in American Public Health Association. 1970. Recommended Procedures for the Examination of Seawater and Shellfish, 4th ed. APHA, Washington, DC., p14-15)

11. Kovacs' reagent (R38)

12. Voges-Proskauer (VP) reagents (R89)

13. Gram stain reagents (R32)

14. Methyl red indicator (R44)

15. Violet red bile agar (VRBA) (M174)

16. VRBA-MUG agar (M175)
17. EC-MUG medium (M50)
18. Lauryl tryptose MUG (LST-MUG) broth (M77)
19. Peptone Diluent, 0.5% (R97)

C. MPN - Presumptive test for coliforms, fecal coliforms and *E. coli*

Weigh 50 g of food into sterile high-speed blender jar Frozen samples can be softened by storing for <18 h at 2-5°C, but do not thaw. Add 450 mL of Butterfield's phosphate-buffered water and blend for 2 min. If <50 g of sample are available, weigh portion that is equivalent to half of the sample and add sufficient volume of sterile diluent to make a 1:10 dilution. The total volume in the blender jar should completely cover the blades.

Prepare decimal dilutions with sterile Butterfield's phosphate diluent or equivalent. Number of dilutions to be prepared depends on anticipated coliform density. Shake all suspensions 25 times in 30 cm arc or vortex mix for 7 s. Using at least 3 consecutive dilutions, inoculate 1 mL aliquots from each dilution into 3 LST tubes for a 3 tube MPN analysis (other analysis may require the use of 5 tubes for each dilution; See IV). Lactose Broth may also be used. For better accuracy, use a 1 mL or 5 mL pipet for inoculation. Do not use pipets to deliver <10% of their total volume; eg. a 10 mL pipet to deliver 0.5 ml. Hold pipet at angle so that its lower edge rests against the tube. Not more than 15 min should elapse from time the sample is blended until all dilutions are inoculated in appropriate media.

Incubate LST tubes at 35±0.5°C. Examine tubes and record reactions at 24±2 h for gas, i.e., displacement of medium in fermentation vial or effervescence when tubes are gently agitated. Re-incubate gas-negative tubes for an additional 24 h and examine and record reactions again at 48±3 h. Perform confirmed test on all presumptive positive (gas) tubes.

D. MPN - Confirmed test for coliforms

From each gassing LST or lactose broth tube, transfer a loopful of suspension to a tube of BGLB broth, avoiding pellicle if present. (a sterile wooden applicator stick may also be used for these transfers). Incubate BGLB tubes at 35±0.5°C and examine

for gas production at 48±3 h. Calculate most probable number (MPN) of coliforms based on proportion of confirmed gassing LST tubes for 3 consecutive dilutions.

E. MPN - Confirmed test for fecal coliforms and *E. coli*

From each gassing LST or Lactose broth tube from the Presumptive test, transfer a loopful of each suspension to a tube of EC broth (a sterile wooden applicator stick may also be used for these transfers). Incubate EC tubes 24±2 h at 45.5°C and examine for gas production. If negative, reincubate and examine again at 48 ± 2 h. Use results of this test to calculate fecal coliform MPN. To continue with *E. coli* analysis, proceed to Section F below. The EC broth MPN method may be used for seawater and shellfish since it conforms to recommended procedures. (Caution: see Note below).

NOTE: Fecal coliform analyses are done at 45.5±0.2°C for all foods, except for water testing and in shellfish and shellfish harvest water analysis, which uses an incubation temperature of 44.5±0.2°C.

F. MPN - Completed test for *E. coli*.

To perform the completed test for *E. coli*, gently agitate each gassing EC tube, remove a loopful of broth and streak for isolation on a L-EMB agar plate and incubate for 18-24 h at 35±0.5°C . Examine plates for suspicious *E. coli* colonies, i.e., dark centered and flat, with or without metallic sheen. Transfer up to 5 suspicious colonies from each L-EMB plate to PCA slants, incubate them for 18-24 h at 35± 0.5°C and use for further testing.

NOTE: Identification of any 1 of the 5 colonies as *E. coli* is sufficient to regard that EC tube as positive; hence, not all 5 isolates may need to be tested.

Perform Gram stain. All cultures appearing as Gram-negative, short rods should be tested for the IMViC reactions below and also re-inoculated back into LST to confirm gas production.

Indole production. Inoculate tube of tryptone broth and incubate 24±2 h at 35±0.5°C. Test for indole by adding 0.2-0.3 mL of Kovacs' reagent. Appearance of distinct red color in upper layer is positive test.

Voges-Proskauer (VP)-reactive compounds. Inoculate tube of MR-VP broth and incubate 48±2 h at 35±0.5°C. Transfer 1 mL to 13 × 100 mm tube. Add 0.6 mL

α-naphthol solution and 0.2 mL 40% KOH, and shake. Add a few crystals of creatine. Shake and let stand 2 h. Test is positive if eosin pink color develops.

Methyl red-reactive compounds. After VP test, incubate MR-VP tube additional 48±2 h at 35±0.5°C. Add 5 drops of methyl red solution to each tube. Distinct red color is positive test. Yellow is negative reaction.

Citrate. Lightly inoculate tube of Koser's citrate broth; avoid detectable turbidity. Incubate for 96 h at 35±0.5°C. Development of distinct turbidity is positive reaction.

Gas from lactose. Inoculate a tube of LST and incubate 48±2 h at 35±0.5°C . Gas production (displacement of medium from inner vial) or effervescence after gentle agitation is positive reaction.

Interpretation: All cultures that (a) ferment lactose with gas production within 48 h at 35°C, (b) appear as Gram-negative nonsporeforming rods and (c) give IMViC patterns of ++-- (biotype 1) or -+-- (biotype 2) are considered to be *E. coli*. Calculate MPN of *E. coli* based on proportion of EC tubes in 3 successive dilutions that contain *E. coli*.

NOTE: Alternatively, instead of performing the IMViC test, use API20E or the automated VITEK biochemical assay to identify the organism as *E. coli*. Use growth from the PCA slants and perform these assays as described by the manufacturer.