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THESIS

CHARACTERIZATION OF RICE BRAN OIL ENCAPSULATION AND ITS APPLICATION IN FOOD MODEL

RATCHANEE CHAROEN

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the Requirements for the Degree of
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Rice bran is an underused coproduce of rice milling. The value is partially captured through extraction and refining of the rice bran oil which contains high nutritional benefits as a mixture of antioxidants and phytosterols. For novel product development, it should be able to use in wide range of food products, not only used as the cooking oil. Therefore, the encapsulation method was selected to study. The objectives of this study were to determine the effect of the oil extraction methods from rice bran, then study the effect of biopolymer type and environmental stresses (pH, salt and heat) on the stability of rice bran oil (RBO) in water emulsion including lipid oxidation during storage. The spray drying was selected to encapsulate the RBO (o/w emulsion) to produce the powder form and the characterization of RBO powder was also investigated. Finally, the utilization of RBO powder was studied. Initially, RBO extracted by cold pressed extraction was selected for the study of the encapsulated RBO due to the safety and the qualities of edible oil including high bioactive content. Biopolymer type had an effect on the stability of RBO in water emulsion under the different environmental stresses. The results indicated that extensive droplet aggregation occurred in whey protein isolate (WPI) stabilized emulsions around their isoelectric point (4<pH<6), at high NaCl (>200 mM), and at high temperatures (>70 °C). There was slightly effect of pH, salt concentration and temperature on emulsions stabilized by gum arabic (GA) or modified starch (MS). WPI or MS stabilized emulsions were stable to lipid oxidation while GA stabilized emulsion was unstable to lipid oxidation which were attributed to the rate of lipid oxidation increased in the following order GA>>WPI≈MS (pH7, with pro–oxidant). When the characterization of WPI or MS stabilized RBO powder was examined, the results showed that the powder had white color with small particle (<25 μm), contained 2.0–2.7% moisture and 30.3–32.7% total fat. The encapsulation efficiency was 92.6–95.2%. From the sorption isotherm study, it was found that the GAB model (R^2 = 0.99) was suitable for prediction of the equilibrium moisture content values. From the study of kinetic reaction of the chemical deterioration of the RBO powder stored at 25, 35 and 45°C for 80 days showed that the reaction order was different between WPI stabilized rice bran oil powder (n = 1) and MS stabilized rice bran oil powder (n = 0). For the activated energy values of the reaction (E_a), powder stabilized with WPI had $E_a$value higher than powders stabilized with MS. The utilization of rice bran oil powder as a coffee creamer found that the sensory properties of rice bran oil powder including color, odor and favor were needed to be improved to be similar to those of coffee creamer in commercial production. This can be done by adding whitening agent, stabilizing salt, favor and color agents etc.

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Ratchanee Charoen

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LIST OF ABBREVIATIONS

°C	=	degree Celsius
h	=	hour
sec	=	second
min	=	minute
g	=	gram
mL	=	milliliter
µL	=	microliter
M	=	molar
mg/L	=	milligram per liter
µm	=	micrometer
mm	=	millimeter
nm	=	nanometer
ppm	=	parts per million
S.D.	=	Standard Deviation
T_g	=	Glass transition temperature
wt%	=	percentage by weight
v/v	=	volume per volume
w/v	=	weight per volume
psi	=	pounds per square inch
mbar	=	millibar
etc.	=	et cetera (and so on)
e.g.	=	*exempli gratia* (for example)

CHARACTERIZATION OF RICE BRAN OIL ENCAPSULATION AND ITS APPLICATION IN FOOD MODEL

INTRODUCTION

Thailand has become the world's largest rice exporter. The average annual production of Thai rice is nearly 34 million tons (The rice exporters association, 2012). Before consumption, all rice is milled at the number of 24.3 million tons of white rice and 2.8 million tons of rice bran. Almost 70% of rice bran is used as animal feed, while only about 20% of rice bran is extracted into rice bran oil (Naivikul *et al.*, 2008). The oil extraction of human consumption is one possible way to use the rice bran waste or utilization of rice bran.

Rice bran oil (RBO) is rich in vitamin E complex, tocopherols, tocotrienols, phytosterols, polyphenols and squalene. It contains a potent antioxidant known as gamma oryzanol (Vieno *et al.*, 2000; Juliano *et al.*, 2005). The presence of high level of natural antioxidants means that RBO has a relatively long shelf life, compared to other cooking oils. The interest in RBO has also been growing because of its potential health benefits. Many studies have shown that RBO reduces "bad" cholesterol (LDL) level without reducing "good" cholesterol (HDL) level (Gunstone *et al.*, 1994; Moreau *et al.*, 2002). RBO contains high level of vitamin E that has been reported to have hypocholesterolemic, anti–cancer and neuroprotective properties (Sen *et al.*, 2007). Recently, it has been shown that gamma oryzanol from RBO can regulate antioxidant and stress genes in rats (Ismail *et al.*, 2010). Consequently, there is an increasing interest in the incorporating RBO into a wide variety of food products in order to benefit from its desirable functional and nutritional characteristics.

Rice bran oil can be extracted by either pressing or solvent extraction. Extraction by pressing is not commonly used because the large quantity of oil (20–30%) still remains in the bran after pressing (Orthoefer and Eastmen, 2004). Solvent extraction is more commonly used because the process leaves less than 1% of residual

oil content. Oil can be extracted by either batch or continuous process and hexane is the most preferred solvent (Orthoefer, 2005). The process of removing the impurities from crude oil also effect on the important substances. The example of the composition of crude oil, refined oil of corn, rape seed, sunflower and soya oil was showed in the term of sterol content (mg/100g). It was reported that crude oil had free sterol more than refined oil (Abidi, 2001; Van Hoed *et al.*, 2006). The comparison of the active ingredient values contained in the rice bran oil extracted by different methods (solvent extraction and cold pressed extraction) is necessary for further selection of high quality of rice bran oil.

The utilization of rice bran oil in food is limited due to its high susceptibility to oxidation. Lipid oxidation can be reduced using microencapsulation of the oil (Augustin and Hemar, 2009). Microencapsulation is a process whereby particles of sensitive or bioactive materials are covered with a thin film of a coating material. The encapsulated substance (e.g. fats, oils, aromas, flavors) is usually referred as the "core" material, whereas the film surrounding the core is usually called the "wall" material (Garti *et al.*, 2007; Wang *et al.*, 2007). Spray drying is the most commonly used microencapsulation method in the food industry as it is both efficient and cost effective. The process generally involves the dispersion of the core material into a polymer solution (e.g., protein, carbohydrates), the formation of an emulsion or dispersion and the atomization of the mixture into the drying chamber (Risch, 1995; Lakkis, 2007). The physicochemical properties of liquid emulsion before spray drying are critical to their ability to encapsulate oils. Obtaining a stable liquid emulsion is a prerequisite for proper encapsulation in spray dried powders (Sagalowicz and Leser, 2010).

Physical properties of food powders (e.g. caking, stickiness, crystallization, dispensability and solubility) can be changed upon the storage (Barbosa–Canovas and Juliano, 2005). The quality of food powders also depends on temperature and moisture. The physical changes of the solid matrix of microencapsulated oil may affect the oil distribution. The partial release of encapsulated oil and the released oil then may be more exposed and undergo rapid oxidation (Kuang *et al.*, 2010). Hence,

the evaluation of the effects of the physical properties on the quality of food powders becomes more complicated. A better understanding of the relationship between physical and chemical properties is essential for future designing of stable encapsulated products.

Therefore, the encapsulated rice bran oil into powder form was explored in this study. The goal is to use the encapsulated technology in order to reduce the deterioration of a substance in rice bran oil by environmental factors. This study starts with the comparison of the quality of rice bran oil through the extraction by different methods, followed by creating high stable emulsion (oil–in–water) using of suitable biopolymers. Then, a spray dry technique is used to encapsulate rice bran oil into dry form (rice bran oil powder). The changes in the quality of rice bran oil powder during storage were also investigated. The results from this study can be the guidelines for the development of rice bran oil powder by consumer survey and sensory evaluation.

OBJECTIVES

1. To compare the qualities of Khao–dawk–mali 105 crude oil and the qualities of mixed–bran crude oil obtained by different methods.

2. To examine the effect of biopolymer types on the emulsion formation and study the influence of environmental stresses on the stability of rice bran oil stabilized by food grade biopolymers.

3. To determine the influence of wall material composition on the formation, stability and physicochemical properties of encapsulated rice bran oil.

4. To explore primary data of the consumer on ready to eat product from rice bran oil and the use of oil powder in food model.

LITERATURE REVIEW

In the development of functional foods, the several approaches, which involve several interrelated elements, functioning in harmony before the product can be successfully launched on the market, should be considered. The process should start with the consideration and decision–making about the product concept and the positioning strategy, in particular on regulatory positioning, labeling, etc. The next step is to identify chemically the bioactive components or ingredients, which are considered to provide the desired beneficial effect on health. In the next step, the feasible technologies with appropriate analytical methods have to be applied or developed the product formulation with the desired health–enhancing properties (Menrad, 2003; Siró *et al.*, 2008). The integrated approach for the development of functional foods also include; (i) to eliminate a component known or identified as causing deleterious health effects, e.g. allergenic proteins; (ii) to increase the concentration of the beneficial natural bioactive component in the food up to the desired, effective level; (iii) to add a component which is not normally present in most foods, but for which beneficial effects have been demonstrated; (iv) to replace a component usually a macronutrient, the intake of which is considered harmful to health in high intake amount, by a component for which the beneficial effect has been demonstrated; (v) to improve the bioavailability of, or to modify food components for which beneficial effects have been demonstrated and (vi) to monitor effectively the amount and the efficacy of beneficial bioactive components (Korhonen, 2002).

1. Encapsulation

Encapsulation is one of the quality preservation techniques to coat or trap solids, liquids or gaseous material in the miniature, sealed capsules, which are capable to release their contents under the specific conditions (Desai and Park, 2005; Kuang *et al.*, 2010). The packaging materials or outer side are called coating–, wall–, carrier or shell material. The packaged materials or inner side can be a pure material or a mixture, which are called coated–, core–, actives material, internal phase or payload (Mozafari, 2006; Fang and Bhandari, 2011). Microencapsulation is small vesicles or

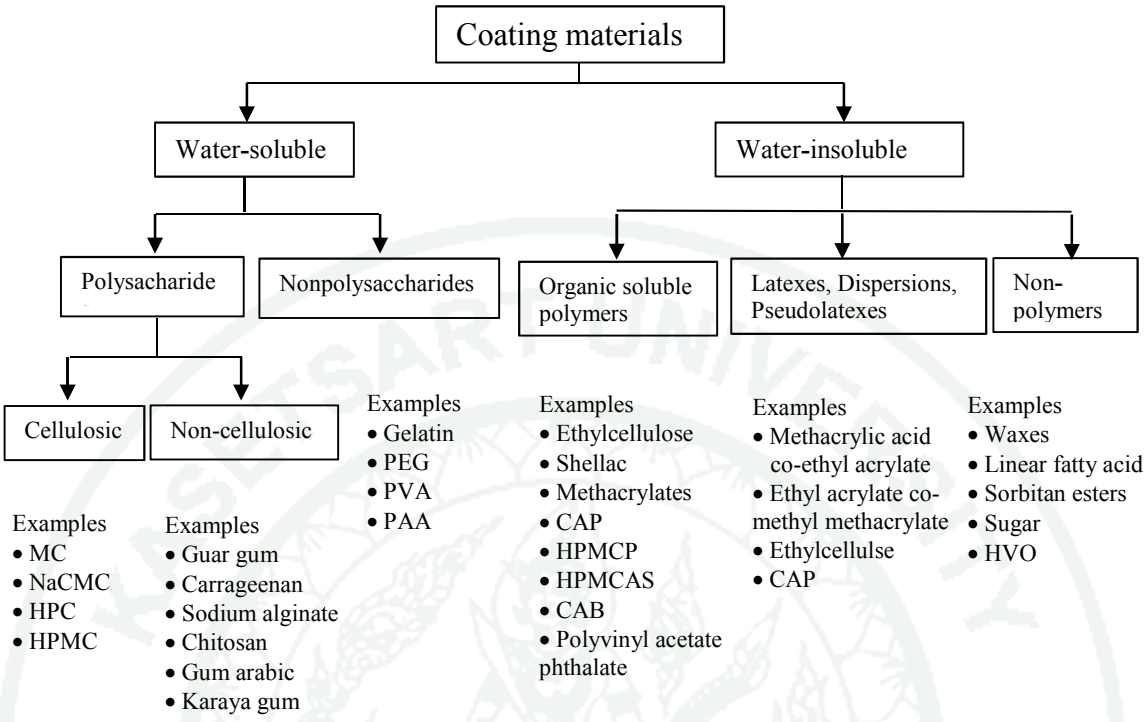
particulates that may range from sub–micron to several millimeters in size (Champagne and Fustier, 2007). The various morphologies can be produced for encapsulation. The two common major morphologies are shown in Figure 1.

Figure 1 Two major forms of encapsulation: the mononuclear capsule (left) and the aggregate (right).

Source: Fang and Bhandari (2010)

Many techniques have been used for encapsulation such as spray drying, spray cooling/chill, extrusion, fluidized bed coating, co–acervation, liposome, entrapment, inclusion complexation, centrifugal suspension separation, lyophilization, cocrystallization and emulsion. There are three steps to encapsulate bioactive substances. The first step is the formation of the wall material around substance to be encapsulated. The second step is making sure that the undesired leakage does not occur and the final step is ensuring that the undesired materials are kept out (Madene *et al.*, 2006; Mozafari, 2006; Champagne and Fustier, 2007).

The type of coating material selected can vary depending on the objective of microencapsulation process (taste masking, enteric protection, time–release) and the desired barrier properties. By the selection of the appropriate coating material, a reservoir system can be designed to protect the bioactive ingredient within the core, until it reaches a favorable biological environment, based on the pH, the temperature, the ionic strength, the enzyme activity or other environmental factors. Figure 2 shows the different categories of coating materials and some common examples. These materials are normally applied to the core by spraying a solution or suspension of the coating material (Felton *et al.,* 1997; Anal *et al.,* 2007; Kuang *et al*., 2010).



Abbreviations: methylcellulose (MC); sodium carboxylmethy cellulose (CMC); hydroxypropyl cellulose (HPC); polyethylene glycol (PEG); polyvinyl alcohol (PVA); polyacrylic acid (PAA); cellulose acetate phthalate (CAP); hydroxypropyl methylcellulose phthalate (HPMCP); hydroxypropylmethyl cellulose acetate succinate (HPMCAS); cellulose acetate butyrate (CAB); hydrogenated vegetable oil (HVO)

Figure 2 Different categories of coating materials and common examples.

Source: Kuang *et al*. (2010)

In addition, a good knowledge of the physicochemical interactions occurring between bioactive compounds and the main constituents of foods such as lipids, polysaccharides (Langourieux and Crouzet, 1994), and proteins (O'Neill, 1996) is required for the food control. Characteristics of the major wall materials used for the food encapsulation are reported in Table 1.

Table 1 Characteristics of the wall material used for encapsulating flavors.

Wall material	Interest
Maltodextrin (DE < 20)	Film forming
Corn syrup solid (DE >20)	Film forming, reductability
Modified starch	Very good emulsifier
Gum Arabic	Emulsifier, film forming
Modifed cellulose	Film forming
Gelatin	Emulsifier, film forming
Cyclodextrin	Encapsulant, emulsifier
Lecithin	Emulsifier
Whey protein	Good emulsifier
Hydrogenated fat	Barrier to oxygen and water

Source: Madene *et al*. (2006)

In the food industry, the microencapsulation process can be applied for a variety of reason, which has been summarized by Desai and Park (2005) as follows:

i) protection of the core material from degradation by reducing its reactivity to its outside environment.

ii) reduction of the evaporation or transfer rate of the core material to the outside environment.

iii) modification of the physical characteristics of the original material to allow easier handling.

iv) tailoring the release of the core material slowly over time or at a particular time.

v) to mask an unwanted flavor or taste of the core material.

vi) dilution of the core material when only small amounts are required, while achieving uniform dispersion in the host material.

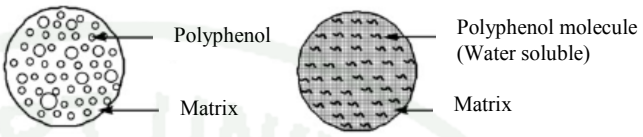
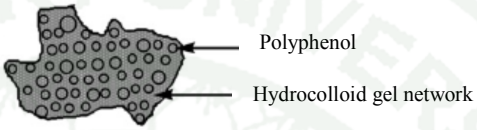
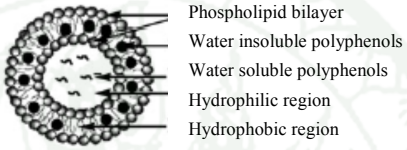
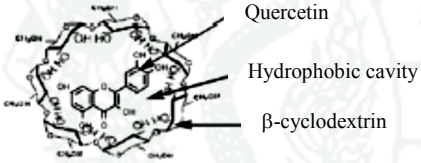
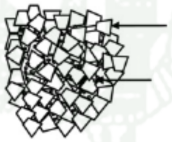
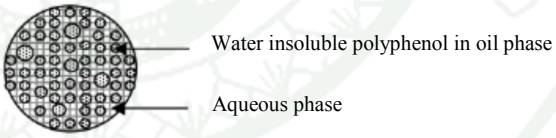
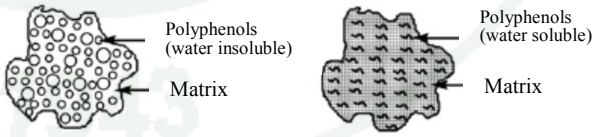
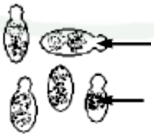
vii) to help separate the components of the mixture that would otherwise react with one another.

According to Fang and Bhandari (2010) have been summarized the technologies of encapsulation of polyphenols, which have been contributed to the functional foods, nutraceutical and pharmaceutical industries due to their potential health benefit to human. This review also shows the characteristics of encapsulated polyphenolic capsule produced by different encapsulation processes (Table 2).

From the literature, it is clear that, instead of free compounds, the utilization of encapsulated bioactive substance can lead to the improvement in both the stability and bioavailability of the compounds in vivo and in vitro (Desai and Park, 2005; Mozafari, 2006; Augustin and Hemar, 2009). The future researches of encapsulated micronutrients are likely to focus on the aspects of the delivery and the potential use of co–encapsulations methodologies, where two or more bioactive ingredients can be combined to have a synergistic effect. With a deep understanding of the health benefits of bioactive compound, the improvements in manufacturing technologies, new strategies for stabilization of fragile nutraceuticals and the development of novel approaches to site–specific carrier targeting, encapsulated substance will play an important role in the increasing of the efficacy of functional foods (McClements *et al.*, 2009; Fang and Bhandari, 2011).

A wide range of micronutrients and bioactive substances has been studied or is now interesting to be added to food products for the potential health–promoting properties (Table 3). Most of them are extracted from plants, fungi, micro algae or marine biomass, for example, plant–derived antioxidative phytochemicals (flavonoids, phytoestrogens, phenolic acids, lignans, phytosterols), prebiotics (oligosaccharides), probiotics (lactic acid bactetiabifidobacteria), peptides, lipids, plant– and animal–derived bioactive proteins, as well as minerals and vitamins. These bioactive substances might help in the prevention of chronic diseases such as cardiovascular diseases and autoimmune diseases. They might also help in the enhancement of the performance and well–being of the individual.

Table 2 Illustration of the characteristics of encapsulated polyphenolic capsule produced by various encapsulation processes.

Encapsulation technology	Illustration of characteristics
Spray drying	
Coacervation	
Liposomes	
Inclusion	
Cocrystallization	
Nanoparticles	
Freeze drying	
Yeast encapsulation	
Emulsion	

Source: Fang and Bhandari (2010)

Table 3 The examples of bioactive food components and their possible physiological target functions or diseases.

Bioactive component	Target function/disease
Phytochemicals	Cardiovascular diseases
flavonoids	Cancer, prevention of cellular
phytoestrogens	Oxidative damage
lignans	
lycopene	
Bioactive lipids	Cardiovascular diseases
omega–3–fatty acids	Arthritis, cancer
conjugated linoleic acid	
Plant sterols	Cholesterol reduction
sitosterol	
stanol esters	
Bioactive proteins and peptides	Hypertension, immune system
lactoferrin	Cancer, osteoporosis
bioactive peptides	
Probiotic bacteria	Digestive tract, immune system,
lactic acid bacteria	Allergy, cancer, cholesterol reduction
bifidobacteria	
Prebiotics	Digestive tract, diabetes, obesity, dental
oligosaccharides	caries
sugar alcohols	
Minerals	Osteoporosis, hypertension
calcium	
Vitamins	Osteoporosis, cardiovascular diseases
folic acid	
tocopherols	

Source: Korhonen (2002)

2. Rice bran and its utilization

Rice bran oil, which also called rice oil, has been used extensively in Asian countries such as Japan, Korea, China, Taiwan, Thailand and Pakistan (Orthoefer, 2005). Recently, rice bran oil become more interesting because it is identified as "healthy oil", which can reduce serum cholesterol in blood (Bidlack *et al.*, 2001; Cheruvanky, 2003). Rice bran oil is a minor constituent of rough rice, compared with the carbohydrate and protein content. Two major classes of lipids are presented: those internal within the endosperm and those associated with the bran. These internal lipids contribute to the nutritional, functional and sensory qualities of rice (Orthoefer, 2005). Rice bran oil production is estimated at 720 thousand metric tons. Major producing countries are shown in Table 4. India is the lead producer, followed by China and Japan (Orthoefer and Eastman, 2004).

Table 4 The production of rice bran oil.

Country	Thousand Metric Tons
Brazil	1.5
Cambodia	4.6
China	90.0
India	472.7
Indonesia	0.15
Japan	65.0
Korea	11.7
Laos	2.6
Burma	17.6
Nepal	7.6
Sri Lanka	5.5
Thailand	7.8
Vietnam	7.6

Source: Orthoefer (2005)

2.1 Rice composition

Rice paddy contains 20% of rice husk, 1–2% of rice germ, 8–9% of rice bran and 70% of rice starchy endosperm (Figure 3). Rice is a staple food due to nutrient content such as protein (5–7%), fat (1.5–2.3%), ash (3–5%), carbohydrate (64–73%) and dietary fiber (16–19%) of a whole grain (Orthoefer, 2005; Nivikul *et al.,* 2008).

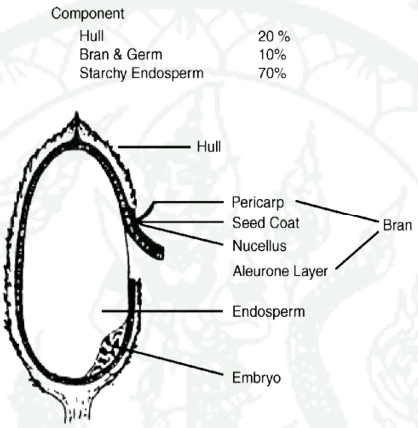


Figure 3 Relative proportion of major rice caryopsis components.

Source: Orthoefer (2005)

The non starch lipids in the aleurone, subaleurone and germ layers are 86–91% of neutral lipids, 2–5% of glycolipids and 7–9% of phospholipids, although these are variable because of different milling degrees (Choudhury and Juliano, 1980; Orthoefer, 2005).

2.2 Rice bran

Rice bran is one of three co–products obtained from rice milling. The other two co–products are milled rice and rice hulls (Orthoefer and Eastman, 2004). Rice bran is a source of dietary fiber, protein and oil. However, only limited quantities are sold for food applications, partially due to its instability (Choudhury and Juliano,

1980). The amount of protein, fiber and ash content found in rice bran with germ are comparable to those of other cereal bran (Table 5).

Table 5 The comparative composition (%) of rice bran and other cereal bran at 14% moisture.

Constituent	Rice bran	Wheat Bran	Corn bran	Rye Bran	Sorghum Bran
Crude protein	12.0–15.6	14.5–15.7	7.8–11.5	14.6	7.7–15.0
Crude fat	15.0–19.7	2.9–4.3	4.4–8.1	2.6	4.6–4.7
Crude fiber	7.0–11.4	6.8–10.4	2.6–9.4	6.6	7.4–9.1
Available–carbohydrates	31.1–52.3	50.7–59.2	58.9–62.6	58.8	54.3–64.1
Crude ash	6.6–9.9	4.0–6.5	1.9–3.4	4.2	2.1–3.0

Source: modified from Orthoefer and Eastman (2004)

2.3 Rice processing

In general, paddy is processed to be white rice. However, rice can be processed into many types of products, which will add value to rice (Figure 4). Thai rice industry can produce some products such as refined rice bran oil and by–product from refining process, defatted rice bran for animal feed, fatty acid and soap. Currently, none of Thai rice company can produce higher value added products such as furfural silicon, tocotrienol, tocopherol, γ–oryzanol and ferulic acid (Nivikul *et al.,* 2008).

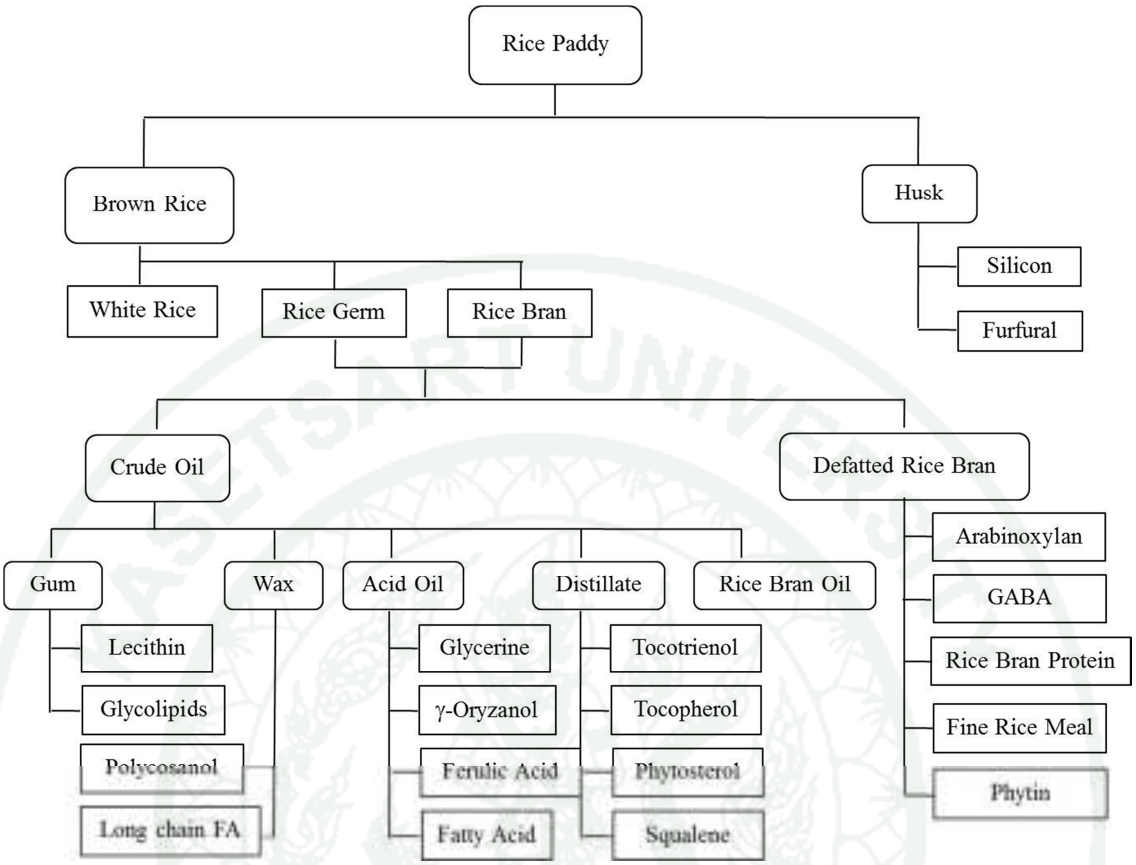


Figure 4 Value added products from rice, rice bran and rice bran oil.

Source: Naivikul *et al.* (2008)

2.4 Rice bran oil compositions and nutritional properties

The fatty acid composition of nonstarch lipids is showed in Table 6. Oil extracted from rice bran contains 20.1% of total lipid, 89.2% of neutral lipids, 6.8% of glycolipid and 4.0% of phospholipid (Shin and Godber, 1996). The oleic, linoleic and palmitic are the major fatty acids in rice bran oil (Orthoefer, 2005; Nivikul *et al.,* 2008).

Monounsaturated fatty acid: MUFA (oleic acid, 18:1) leads to a reduction in the LDL cholesterol and the increase in the HDL cholesterol. This results in an effect of reducing the risk of atherogenesis. Oleic acid thus participates in the

protecting against cardiovascular diseases (CVD). The diets with high in MUFA also lower the concentrations of circulating triacylglycerols (Berry *et al.*, 1992; Ashton *et al.*, 2001). Linoleci acid (18:2, n–6) is an essential fatty acid that typically presents (~34%) in rice bran oil. It is the precursor of the metabolic series of the PUFA known as omega–6 (ω–6), which is the essential fatty acid for synthesis of eicosanoids (controlling platelet aggregation, inflammatory and immune phenomena). A higher intake of linoleic acid also protects against stroke, possibly through the potential mechanism of the decreased blood pressure (Iso *et al.*, 2002).

Table 6 Major lipid classes of crude bran oil extracted from raw rice bran and their fatty acid composition.

Lipid class[a]	wt%	Fatty acid composition (%)								
		14:0	16:0	18:0	18:1	18:2	18:3	20:0	saturated	unsaturated
TL	20.1	0.4	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

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

Source: modified from Orthoefer (2005)

According to the National Cholesterol Education Program (NCEP), the optimum intake of fat for an adult is 25–35% (normally ~30%) of its total caloric intake. Therefore, for an adult man consuming 2000 calories in diet, he should consume about 600 calories (30%) from fat per day. Within these30% of calories, NCEP recommends a diet that provides less than 7% of calories from saturated fatty acid (SFA), up to 10% from polyunsaturated fatty acid (PUFA), and up to 20% from monounsaturated fatty acid (MUFA) (Franz, 2003). In comparison to other types of

vegetable oil, rice bran oil is closely matched to the NCEP recommendation of fat (Figure 5).

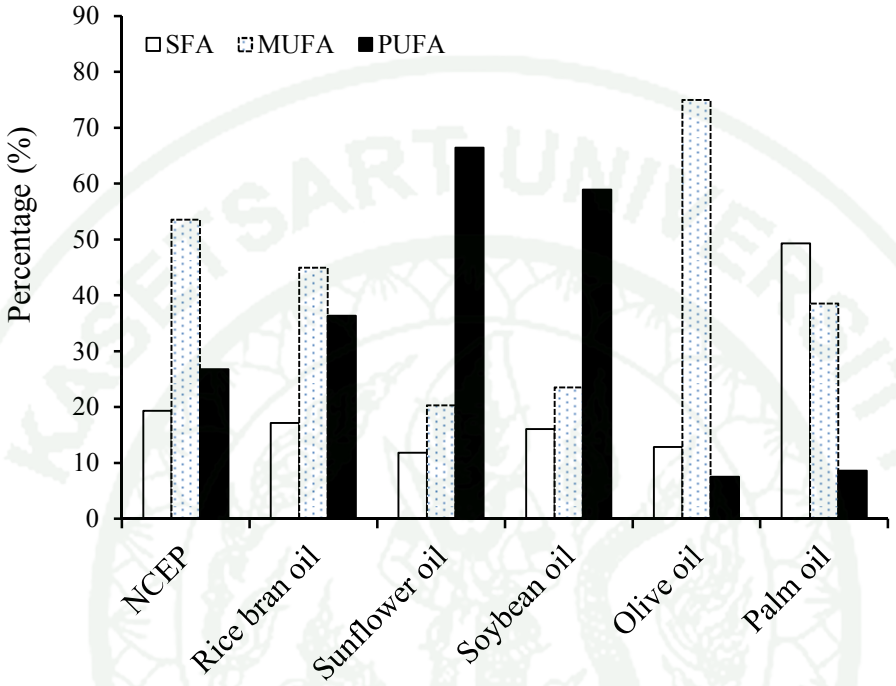


Figure 5 Balance of fats in some edible vegetable oil.

Source: modified from Naivikul *et al.* (2008)

Oryzanol: a component of rice bran oil, that plays an important role as nutraceutical compound, is γ–oryzanol (Rukmini and Raghuram, 1991). Oryzanol is about 1.5–2.9% of rice bran oil. It has melting point of 138.5 Cº. Oryzanol is now known as a mixture of steryl and other triterpenyl esters of ferulic acid (Figure 6). The oryzanol content is dependent on rice grain variety with long grain rice at 6.24 mg/g and medium grain rice at 5.17 mg/g (Lloyd *et al.*, 2000; Godber and Juliano, 2004). The sterol components of γ–oryzanol are primarily campesterol, sitosterol, cycloartenol and 24–methylene cycloartanol (Orthoefer, 2005).

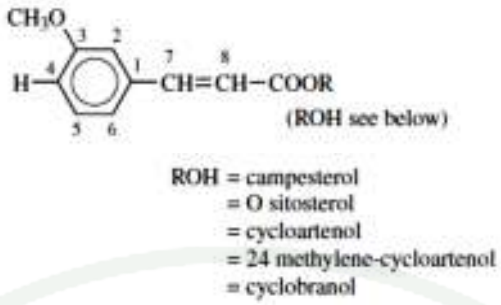


Figure 6 Major ferulates in oryzanol.

Source: Godber and Juliano (2004)

Oryzanol has been suggested to have potential functionality such as the antioxidant activity (Nanua *et al.*, 2000; Juliano *et al.*, 2005), the free radical scavenging (Akiyama *et al.*, 2001), the reduction of serum cholesterol, the reduction of cholesterol absorption, the decrease of early atherosclerosis and the inhibition of tumor promotion (Sasaki *et al.*, 1990; Rukmini and Raghuram, 1991).

Phytosterol: phytosterols are plant–derived sterols that are structurally similar to cholesterol in vertebrate animals (Figure 7). The chemical structures of phytosterols differ from that of cholesterol because of the presence of modified side chains at carbon C–24. In each dietary phytosterol intake in many foods, sitosterol is the most abundant form of phytosterol (65%), followed by compesterol (30%) and stigmasterol (3%) (Piironen *et al.*, 2000; Moreau *et al.*, 2002).

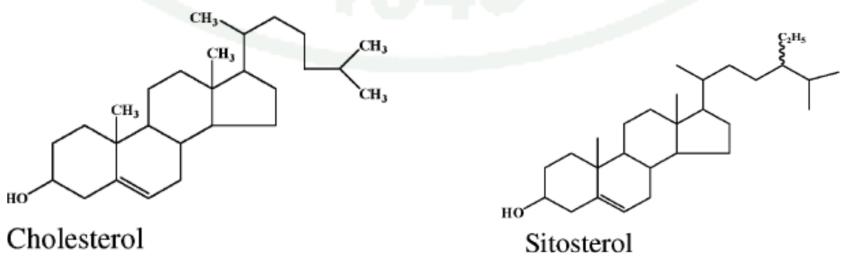


Figure 7 Structure of cholesterol and sitosterol.

Source: Moreau *et al.* (2002)

In general, vegetable oils and products derived from oils are regarded as the richest natural sources of phytosterols. The most significant exception is rice bran oil which contains the highest amount of sterols (10.55 g/kg), followed by corn (7.15–9.52 g/kg) and rapeseed oil (2.50–7.31 g/kg) as shows in Figure 8 (Piironen *et al*., 2000).

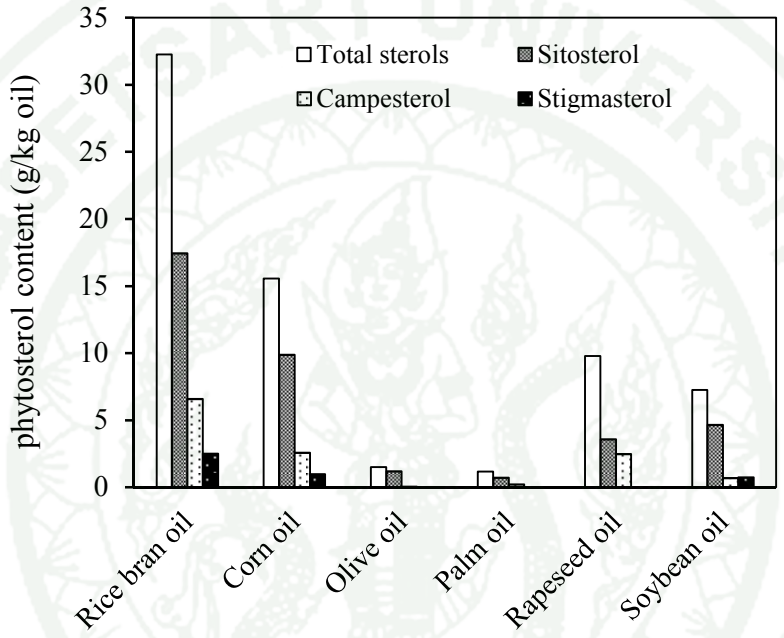


Figure 8 Phytosterols content in various vegetable oils.

Source: Piironen *et al*. (2000)

The evidence from the studies over the past 40 years has shown that the circulating total LDL cholesterol level can be reduced by the ingestion of phytosterols. This reduction is more obvious with consumption of saturated phytosterols such as sitostanol versus less saturated β–sitosterol or compesterol. The mechanisms of hypocholesterolemic action are the inhibition of cholesterol absorption and the decreased excretion from the liver (Ling and Jones, 1995).

Tocopherol and tocotrienols (tocols): in 100 g of crude rice bran oil, it contains 19–46 mg of α–tocopherol, 1–3 mg of β–tocopherol, 1–10 mg of γ–tocopherol, 0.4–0.9 mg of δ–tocopherol, 14–33 mg of α–tocotrienol and 9–69 mg γ–tocotrienol (Kato *et al.*, 1983; Orthoefer, 2005). The chemical structures of tocols are shown in Figure 9. The storage and extraction method of rice bran effect on rice bran stabilization and the concentration of tocols in the oil (Orthoesfer, 2005). Compared with other tocols, γ–tocotrienol is more stable and persists to a greater extent during storage (Shin, *et al.*, 1997).

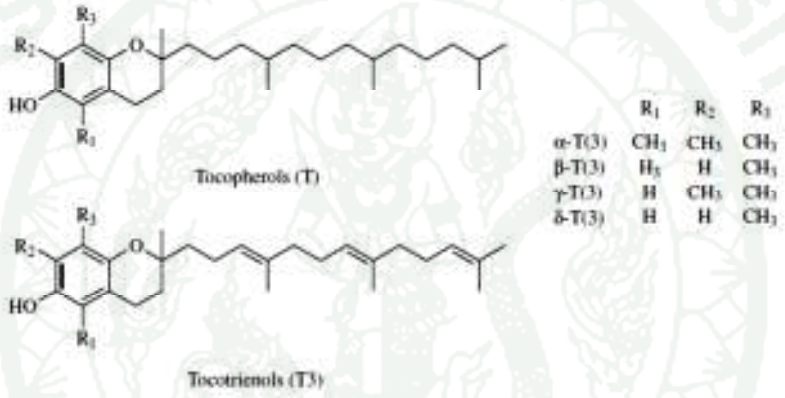


Figure 9 Structure of tocopherol and tocotrienol.

Source: Godber and Juliano (2004)

Tocopherol and tocotrienol are also well recognized as natural antioxidant for their effective inhibition of lipid oxidation in foods and biological systems. The high fractions isolated of tocols have significant health benefits through the modulation of physiological functions that include various atherogenic lipid profiles and antioxidants in hyperlipidemic rats (Minhajuddin *et al.*, 2005). Moreover, tocotrienols lower total cholesterol and LDL cholesterol plasma levels in hamster and in human pilot study (Shin *et al.*, 1997). The ability to prevent cardiovascular disease, coronary heart disease and cancer of tocopherols and tocotrienols by arresting radical damage has been also suggested (Meydani *et al.*, 1986).

2.5 Rice bran to rice bran oil

Earlier methods for recovering the oil use hydraulic pressing (Orthoefer, 2005). In Japanese system for pressing, the raw bran is cleaned by sifting and air classification to remove whole and broken grains and hulls, and to recover rice germ. The bran is then steam cooked, dried, prepressed and finally expeller pressed. The process flow to extract oil from rice bran is shown in Figure 10.

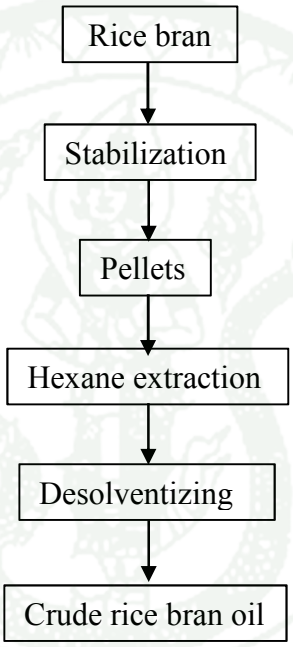


Figure 10 Process for rice oil production.

Source: Orthoefer (2005)

Hexane extraction may be batch or continuous type (Choudhury and Juliano, 1980; Orthoefer and Eastmen, 2004). The bran in the most efficient systems is stabilized, pelletized, and dried (if required). After the pretreated bran is placed in the extractor, hexane is pumped in and allowed to percolate through the bran to extract the oil. The miscella (solvent plus oil) is passed through the filters to remove the bran fines before the evaporation for solvent and crude oil recovery. The production of fines from expander stabilized bran depends on stabilization condition

(Randall *et al.*, 1985). The composition of crude rice bran oil produced by hexane extraction of stabilized bran is shown in Table 7.

Table 7 Crude rice bran oil composition.

Lipid type		Percent
Saponifiable lipids		90–96
	Neutral lipids	88–89
	Triacylglycerols	83–86
	Diacylglycerols	3–4
	Monoacylglycerols	6–7
	Free fatty acids	2–4
	Waxes	6–7
	Glycolipids	6–7
	Phospholipids	4–5
Unsaponifiable lipids		4–2
	Phytosterols	43
	Sterol esters	10
	Triterpene alcohols	28
	Hydrocarbons	18
	Tocopherols	1

Source: Orthoefer (2005)

The instability of rice bran has been associated with lipase activity. As long as the kernel is intact, lipase is physically isolated from lipids (Randall *et al.*, 1985; Orthoefer, 2005). Even dehulling disturbs the surface structure allowing lipase and oil to mix. Oil in intact bran contains 2–4% of free fatty acids. Once bran is milled from the kernel, a rapid increase in the free fatty acid occurs. In high humidity storage, the rate of hydrolysis is 5–10% per day and about 70% in a month as shown earlier (Orthoefer, 2005). The main objective of rice bran stabilization is to arrest lipase and lipoxygenase activity.

Lipase activity results in hydrolysis rancidity. There is a little or no change in flavor of the bran with an increase in FFA (Orthoefer, 2005). Lipoxygenase activity, however, increases with the presence of FFA, resulting in oxidative rancidity (Galliard, 1989). It is oxidative deterioration, which is responsible for the flavor and odor of rancid rice bran. Peroxidase is used as a convenient index of lipase activity. The inactivation temperatures for lipases and associated enzymes depend on the moisture content. At 4% of moisture, the inactivation temperatures for lipoxygenase, lipase and peroxidase are 40 ºC, 55 ºC and 70 ºC, respectively (Orthoefer, 2005). Methods for stabilization of rice bran including dry heating, wet heating and extrusion have been reviewed. Compared with dry heating, wet heating is more effective for bran stabilization for oil extraction. Lipase is inactivated in 3 minutes at 100 ºC (Sayre *et al.*, 1982). The equipments, that can be used, are steam cookers, blanchers, autoclaves and screw extruders with the injected steam and water (Randall *et al.*, 1985). Parboiling of rice is also an example of wet heat stabilization. The lipase in rough rice is inactivated completely by either autoclaving for 3–10 minutes or parboiling. Other stabilization methods, that have been investigated, are refrigeration to reduce the rate of hydrolysis (Orthoefer and Eastman, 2004), lowing pH to reduce lipase activity and chemical additions such as sodium metabisulfite (Orthoefer, 2005; Cheruvanky, 2003).

3. Biopolymer selection

The development of biopolymer systems for the encapsulation of bioactive compound is important for food industry. Many beneficial food bioactives such as ω–3 fatty acid, carotenoids, fat–soluble vitamins and phytosterols are lipophilic. The hydrophobic nature of these compounds makes their incorporation into aqueous foods and beverages challenging (Mozafari, 2006; Champagne and Fustier, 2007). In addition to incorporation problems, many of these lipophilic compounds are also chemically unstable and tend to degrade during storage when incorporated into foods (Madene *et al.*, 2006; Lakkis, 2007). The use of biopolymers such as proteins and polysaccharides to encapsulate and protect these bioactive is thus highly desirable. At present, the understanding of how to create biopolymer particles with specific

functional attributes is still rather limited so a better understanding of structure–function relationship is needed. Identification of the most appropriate ingredients and conditions required to create these particles requires the knowledge of the molecular and functional characteristics of the biopolymers used, as well as the physicochemical mechanisms underlying particle formation (McClements *et al.*, 2009).

In addition to the biopolymers present within a particle, these will usually be other components present, such as water, lipids, minerals and sugars. Some of these components may be essential for assembling the biopolymers into particles, and may have other functions (e.g., lipid droplets trapped within the biopolymer matrix may act as a reservoir for lipophilic components). To form biopolymer particles, the selection of particular proteins, polysaccharides and other components depends on a number of factors; (i) the ability of the components to be assembled into particles; (ii) the functional requirement for the particles (e.g., size, charge and stability to environmental conditions); (iii) legal status, cost ease of use and consistency of the ingredients and processing operations (Willats *et al*., 2006; McClement *et al*., 2007; McClements, 2010).

3.1 The formation of biopolymer

Many types of biopolymers are capable for forming biopolymer nanoparticles or microparticles that can be used to incorporate lipophilic compounds (Figure 11). These particles can be formed from the individual biopolymer types or from mixed biopolymer systems depending on the fabrication mechanism. Biopolymer particles can often be formed from aqueous solution containing a single biopolymer by promoting self–association (Figure 11a). To encourage of self–association, solution conditions are altered such that biopolymer–biopolymer interactions are favored over biopolymer solvent interactions (Figure 11b–d).

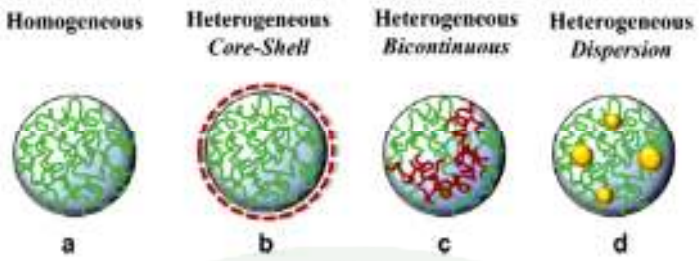


Figure 11 Examples of some different kinds of internal structures that may be formed within biopolymer particles.

Source: McClements (2005)

3.2 Protein selection

Protein is biological polymers comprised of amino acids that come in a variety of different general structures (Figure 12) e.g., random coil, fibrous and globular protein (Belitz *et al.*, 2009). The molecular structure adopted by a particular protein depends on its amino acid sequence, prevailing environmental conditions, e.g., exposure to different temperatures, pressures, solvents, pH values, and ionic compositions (Phillips *et al.*, 1994).

Figure 12 Simplistic schematic representation of different structures that protein and polysaccharide molecules may have in solution.

Source: Belitz *et al.* (2009)

Proteins in milk can be conveniently divided into two major categories: caseins (~80 wt%) and whey protein (~20 wt%). A variety of milk protein ingredients are available for utilization as emulsifiers in food, including whole milk, whey protein and caseins (Dickinson, 1999). These ingredients are usually sold in a powdered form that is light cream–to–white in appearance and has a bland flavor and the powders are normally available in the form of protein concentrates (25–80% protein) or protein isolates (>90% protein). There are relatively large number of different types of proteins in both casein and whey, and that is possible to fractionate these proteins into individual purified fractions (e.g., β–lactoglobulin, α–lactalbumin, bovine serum albumin, β–casein). Whey protein isolate is one of the emulsifiers frequently used in foods because of its ability to facilitate the formation and stabilization of oil–in–water emulsions (Phillips *et al*., 1994; Dickinson, 1997; McClements, 2005). The ability of whey protein to form stable emulsions depends on emulsion composition (including pH and mineral content, salt, sugar, surfactant and polysaccharide contents) and environmental conditions (temperature and pressure) (Kinsella, 1984; Dickinson, 1997; Dalgleish, 1997; Dematriades *et al*., 1997a, 1997b; Singh and Ye, 2000). Whey proteins are therefore suitable for application in food emulsions where the composition and environmental conditions favor a stable product but not in those products where the condition promote emulsion instability.

The number of factors must be considered when selecting a suitable protein or combination of proteins on delivery systems. Firstly, it usually requires the knowledge of specific physicochemical characteristics of the protein involved, such as thermal denaturation temperatures (for globular proteins), helix–coil transition temperatures (for globular proteins), isoelectric points (pI), and sensitivities to specific mono valent or multivalent ions. Secondly, it is often important to establish the electrical characteristics of the protein molecules involved. This is because the electrostatic interactions are often utilized in structure formation, which can be conveniently described by the ζ–potential versus pH profile. Thirdly, it is usually important to have the knowledge of the nature of the biopolymer particles that can be formed after protein association, such as their morphology (fibrous, globular),

physical properties (density, refractive index), size, charge and stability (e.g., pH, ionic strength, temperature and enzymes) (LaClair and Etzel, 2010).

3.3 Polysaccharide selection

Polysaccharides are classified as either homo–polysaccharides (consisting of only one type of mono–saccharide) or hetero–polysaccharides (consisting of different types of monosaccharide). Polysaccharides differ from one to another chemically in terms of the type, number, sequence and bonding of the monosaccharide within the polymer chain (Belitz *et al.*, 2009). The factors must be considered when selecting a suitable polysaccharide or combination of polysaccharides to delivery system. It is important to know the physicochemical properties of the polysaccharide.

Gum arabic is the most commonly used biopolymer emulsifier in food products. It is derived from the natural bark exudate of *Acacia senegal* and consists of at least 3 high–molecular–weight biopolymer fractions. The surface–active fraction is believed to consist of branched arabinogalactan blocks attached to a polypeptide backbone (Anderson *et al*., 1985; Phillips and Williams, 1995; Jayme *et al*.,1999). The hydrophobic polypeptide chain is believed to anchor the molecules to the droplet surface, while the hydrophilic arabinogalactan blocks extend into the solution, providing stability against droplet aggregation through steric and electrostatic repulsion (Phillips and Williams, 1995; Jayme *et al*., 1999). Gum Arabic is an effective emulsifier because of its high water solubility, low solution viscosity, good surface activity and ability to form a protective film around emulsion droplets. However, the relatively high cost, large quantity required and problems associated with obtaining a reliable source of consistently high–quality gum arabic have led many food scientists to investigate alternative sources of biopolymer emulsifiers for use in food (Kim *et al*., 1996; Garti 1999).

Hydrophobically modified starches have been identified as one of the most promising replacements for gum arabic (Trubiano, 1995). The modified starch

used in this study (Purity Gum Ultra; National starch, Bridgewater, NJ, USA) is and octenyl succinate derivative of waxy maize. It consists primarily of amylopectin that has been chemically modified to contain nonpolar side groups. These side groups anchor the molecule to the droplet surface, while the hydrophilic starch chains protrude into the aqueous phase and protect droplets against aggregation through steric repulsion. Modified starch is mildly anionic in aqueous solutions and has a surfaced activity that is almost as high as that of gum arabic (Ray *et al*., 1983).

Interfacial activity: after a biopolymer ingredient has been dissolved adequately in the aqueous phase, it is important to ensure that the solution and environment condition (e.g., pH ionic strength, temperature and solvent composition) will not promote droplet aggregation during homogenization or after emulsion is formed (Dickinson, 2003; McClements, 2005). When a biopolymer adsorbs to an interface, it can adopt a conformation where the non–polar groups are located in the oil phase (away from the water) and the hydrophilic groups are located in the aqueous phase (in contact with the water). The conformation of biopolymer at an interface, and the physic–chemical and structural properties of the interfacial layer formed depend on its molecular structure and interactions (Norde, 2003). Flexible random–coli biopolymers adopt an arrangement, where the predominantly non–polar segments protrude into the oil phase, the predominantly polar segments protrude into the aqueous phase and the neutral regions lie flat against the interface (Figure 13). Random–coil biopolymers are relatively flexible molecules so they can rearrange their structures fairly rapidly. On the other hand, the globular biopolymers are more rigid molecules so they rearrange more slowly. The unfolding of globular protein at an interface often exposes the amino acids that were originally located in the hydrophobic interior of the molecule. This can lead to enhance the interactions with neighboring protein molecules through hydrophobic attraction or disulfide bond formation. Consequently, the globular proteins tend to form relatively thin and compact layers that have high viscoelasticities. This may account for the fact that layers formed by globular protein are more resistant to rupture than those formed by more random–coil protein (Norde, 2003; McClements, 2005).

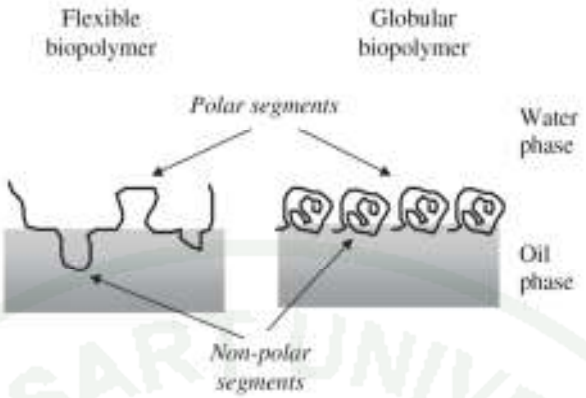


Figure 13 The structure of the interfacial membrane depends on the molecular structure and interactions of the surface–active molecules.

Source: Norde (2003)

4. The stability of oil–in–water emulsion and encapsulated powder

4.1 Emulsion formation and stability

In foods, the two immiscible liquids are usually an oil phase and a watery phase. Emulsions can be classified to the relative organization of the two immiscible phases (Figure 14) (McClements, 2005). A system consisting of oil droplets dispersed in a water phase is called an oil–in–water (O/W) emulsion (e.g., milk, salad dressing, mayonnaise and coconut milk), whereas a system consisting of water droplets dispersed in an oil phase is called a water–in–oil (W/O) emulsion (e.g., butter or margarine). The material within the droplets is usually referred as the dispersed or internal phase, whereas the material within the surrounding liquid is called the continuous or external phase. It is also possible to form more complex arrangements of oil and water within a single system, e.g., W/O/W or O/W/O "double" emulsions (Garti and Bisperink, 1998; Garti and Leser, 2001). These double emulsions are useful for protecting labile ingredients, controlling the release of specific ingredients and for formulating low–fat products.

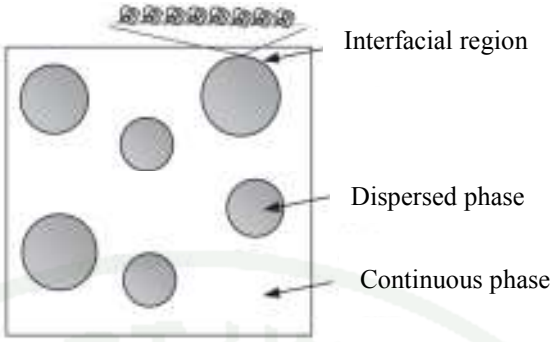


Figure 14 Emulsions consist of liquid droplets (disperse phase) suspended in an immiscible liquid (continuous phase).

Source: McClements (2005)

The physical instability of an emulsion may occur through a number of different processes including creaming, sedimentation, flocculation, coalescence, partial coalescence, ostwald ripening and phase inversion (Figure 15).

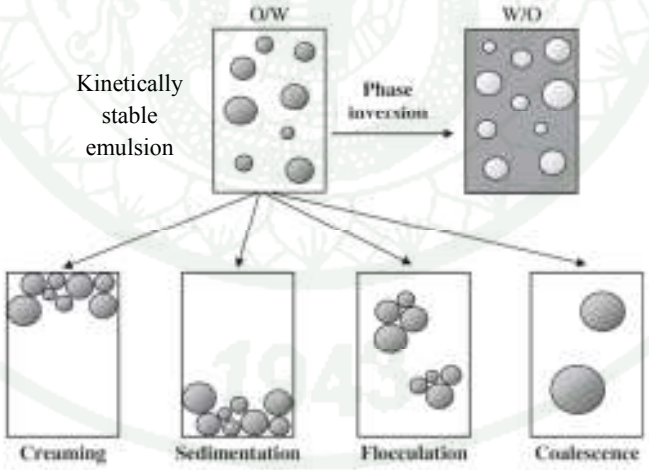


Figure 15 Summary of the physicochemical mechanisms that typically cause instability in food emulsions. The dominant mechanism in particular emulsion depends on its composition, structure and environment.

Source: McClements (2005)

In many food applications, oils are present in the form of lipid droplets dispersed within an aqueous medium (oil–in–water emulsions), rather than as bulk oils (Dickinson, 2002; McClements, 2005). In these systems, the oil droplets remain both physically and chemically stable throughout the shelf life of the product. The relatively high level of functional and nutraceutical components presented within rice bran oil may mean that it behaves differently in emulsions compared to the other edible oils. The presence of minor components within edible oils can impact emulsion performance, due to their ability to impact the physical or chemical stability of these systems (Khan and Shahidi, 2000; McClements, 2005; Arima *et al.*, 2009). For example, some minor components, which may be surface active can interfere with emulsion formation and stability of emulsion by competing with emulsifiers. One of the research objectives is therefore to investigate whether rice bran oil can be used to form stable oil–in–water emulsions, which are suitable for application in a variety of food products.

Ultimately, it would be desirable to produce rice bran oil emulsions that could be incorporated into final products with "consumer–friendly" labels, and so the biopolymer emulsifiers were utilized (rather than synthetic surfactants) for their stabilization. Three different surface–active biopolymers, that have been shown previously to stabilize oil–in–water emulsions including gum arabic, modified starch, and whey protein isolate (Chanamai and McClements, 2002), were selected. Each type of biopolymer emulsifier has different abilities to form and stabilize emulsions. During homogenization, globular proteins tend to adsorb to oil droplet surfaces more rapidly than polysaccharides, hence they are capable for forming smaller droplets (McClements, 2005). In addition, they are usually used at much lower emulsifier–to–oil level than gum arabic or traditional modified starch emulsifiers (Chanamai and McClements, 2002). On the other hand, the globular proteins tend to stabilize emulsions primarily through electrostatic interactions so they are sensitive to the changes in pH and salt (Chanamai and McClements, 2002). The gum arabic and modified starches tend to stabilize emulsions primarily through steric interactions so they are less influenced by pH and salt (Wilde, 2000; Dickinson, 2003; McClements, 2005). In addition, the globular proteins unfold when they are heated above their

thermal denaturation temperature. This may promote emulsion instability through an increase in their surface hydrophobicity (Kim *et al.*, 2002, 2005). On the other hand, polysaccharide emulsifiers tend to be less influenced by thermal treatment (Chanamai and McClements, 2002). Therefore, the influences of pH, ionic strength, and heating on the stability of the biopolymer–stabilized rice bran oil droplets were examined here, since the food emulsions typically experience the variations in these parameters in commercial products.

Rice bran oil has a relatively high concentration of unsaturated fatty acids so it is susceptible to lipid oxidation. The oxidative deterioration of lipids affects the quality of foods, flavor, odor, and nutritive value (Frankel *et al.*, 2002). The rate at which oxidation takes place is dependent on several factors such as the molecular structure of the lipids, the storage temperature, the presence of pro–oxidants and antioxidants, and the structural organization of the lipids (Nawar, 1996; McClements and Decker, 2000). Lipids are often present in food products in the form of emulsions. Many common food products exist as oil–in–water emulsions (e.g., beverages, dressings, sauces, soups, and deserts). In these products, the lipid portion is dispersed as miniscule droplets within an aqueous continuous phase (McClements, 2005). The susceptibility of these emulsified lipids to oxidation depends not only on the factors mentioned previously, but also on the surrounding molecular environment and the interactions with other molecules within their immediate vicinity (Kellerby *et al.*, 2006). Many studies have highlighted the importance of transition metal catalysis as a major factor, responsible for promoting lipid oxidation in the emulsion systems (Waraho *et al.*, 2009; Berton *et al.*, 2011; Guzun–Cojocaru *et al.*, 2011). Iron, a transition metal, is a strong pro–oxidant that is ubiquitous in food systems. Transition metals, which are in close proximity to surface–active lipid hydroperoxides at the lipid droplet interface, will promote hydroperoxide degradation. Iron can decompose hydroperoxides (LOOH) into alkoxyl ($LO^{\bullet}$) and peroxyl ($LOO^{\bullet}$) radicals. In lipid systems, these highly reactive radicals abstract hydrogen from unsaturated fatty acids (LH) within their immediate vicinity to form new radicals that can further promote oxidation and eventually lead to rancidity. The ability of iron to breakdown lipid hydroperoxides depends on its physical location relative to the surface of the

emulsified lipid phase, since the oxidation usually occurs at the oil–water interface (Demetriades *et al.*, 1997a). This ability may be either hindered or promoted, due to the presence of an adsorbed emulsifier layer at the lipid droplet surfaces (Mancuso *et al.*, 2000).

4.2 Encapsulation and powder properties

Emulsion solution through the selection of biopolymer with the highest stability is the encapsulated using the spray dry technique, which changes the rice bran oil into powder dry form.

Spray–drying is well established technology which is available and the most commonly used for encapsulation process in food industry. This method has paved the way for the production of powder with high storage stability. It is also easy handling for some applications, and minimized transportation weight in the comparison with liquid concentrates (Risch, 1995; Soottitantawat *et al*, 2005; Bhandari, 2008). There are many advantage of spray–drying; the ability to produce dry powder very quickly, the product ready for packaging and also can be used to dry a wide range of liquid materials (Bhandari, 2008).

Bioactive compounds (tocopherol, oryzanol, phytosterol) in rice bran oil are not stable to heat during refining oil process (Orthoefer and Eastman, 2004; Orthoefer, 2005). The differences in the stability of sterol compounds depend on the processing conditions, and the presence of other components in the environment (Oehrl *et al*., 2001; Soupas *et al*., 2005). Indeed, it was reported that there are the deterioration of the bioactive compounds in fat or fat products composition such as the deterioration of the vitamin E, the deterioration of oryzanol in the seed oil and the deterioration of phytosterol (Oehrl *et al*., 2001; Soupas *et al*., 2004). The risk of the deterioration of the bioactive compound starts from the process of refining oil until the food process using the heat. Furthermore, the storage stability is more depending on the environment factor such as temperature, light, oxygen and water activity (a_w). In general, moisture and temperature are the two important parameters, affecting rate

of reaction in foods. Until the 1980s, the influence of moisture on chemical reaction, in particular the reactions to food stability, had been described in terms of water activity (Labuza, 1975; Duckworth, 1981; Roos and Karel, 1991a).

Water activity has been considered to be more important than the total amount of water, concerning to quality and stability of food stuff. Water sorption isotherms are the important thermodynamic tools for predicting the interactions between water and food components. They describe the relationship between water activity and the equilibrium moisture content of a food product. They also provide the useful information for food processing operations such as drying, packaging and storage (Lomauro *et al.*, 1985; Labuza and Hyman, 2001). Sorption isotherms are described generally by mathematical models based on empirical or theoretical criteria. The most commonly used equations are Brunauer–Emmett–Teller (BET: equation 1) and Guggen–Heim–Anderson–de Boer (GAB: equation 2). Once some theoretical backgrounds are obtained, their parameters provide a physical meaning related to the sorption process, as compared to empirical models (Aviara *et al.*, 2004; Tonon *et al.*, 2011).

$$X_e = \frac{X_m C_{BET} a_w}{(1-a_w)(1-a_w+C_{BET}a_w)} \quad (1)$$

$$X_e = \frac{X_m G_{GAB} K_{GAB} a_w}{[(1-K_{GAB}a_w)(1-K_{GAB}a_w+C_{GAB}K_{GAB}a_w)]} \quad (2)$$

where X_e is equilibrium moisture content (g water/g dry matter), X_m is monolayer moisture content (g water/g dry matter), a_w is water activity, C_{BET} is constant of equation (1), K_{GAB} is constant of equation (2) and G_{GAB} is constant of equation (2).

In this study, sorption isotherm data was modeled according to BET and GAB models using the solver algorithm and the goodness of fit was evaluated by the determination coefficient (R^2).

Recently, a new alternative approach based on glass transition theory is used to consider the effect of the state of the system on the reaction. The physical stability of amorphous foods has been related to the change from the glassy state to a rubbery state occurring over a temperature range known as the glass transition. A characteristic point of such a range, usually the onset temperature, is taken as the reference glass transition temperature (T_g) (Roos and Karel, 1991b; Sá and Sereno, 1994). As the temperature increases above T_g, the various changes including an increase of free volume and specific heat, an decrease of viscosity and some physical changes (such as collapse, loss of shape, shrinkage or stickiness) may occur during food processing and storage (Schebor *et al.*, 1999; Moraga *et al.*, 2004). It is also possible that the properties of the state of the system (glassy or rubbery system) may contribute to the differences in chemical reaction rates in each of these states.

As mention above, the environment factors affect the quality of the product. Some of those reactions tend to degrade foods during storage. Thus, it is important to know the "temperature dependence" of the rate of such reactions in order to predict product shelf life. The most common assumption is that temperature dependence of the rate of deterioration will follow the well–known Arrhenius relationship (equation 3). It has been stated that the Arrhenius model is applicable for describing the temperature dependence of reactions within a food matrix in the glassy state (Labuza, 1984).

$$k = k_0 e^{-(E_A/RT)} \qquad (3)$$

Under the objective of the present work, the rice bran oil encapsulated powder stabilized with whey protein isolate is compared to the modified starch in order to investigate how the effect of the different carrier agents on the physical properties of powder. In fact, this work provides the experimental data of water sorption of rice bran oil powder. Sorption isotherms were modeled according to BET and GAB models. Furthermore, the kinetics of lipid oxidation of rice bran oil powder, the degradation of the oil in powder and the physical change resulting from the glass

transition were determined experimentally in order to obtain the useful information about powder stability.

5. The use of non–dairy creamer as food model

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 and therefore are commonly described as not being dairy products (although may contain a milk–derived protein). There are some different in nondairy creamers composition e.g., fat, sugar and flavors. The coffee creamers are offered in three different forms: powdered, liquid and the concentrate which comes in a portion controlled by a piece of bag, or pump bottle.

Non–dairy creamers normally contain fat (20–40 wt%), protein (either skim milk solids, more usually sodium casemate, about 6–10%), and carbohydrate (e.g., corn syrup solids). The ingredients are normally formulated as an emulsion which is subsequently dried, preferably spray–dried, and solid in the form of a powder. In non–dairy creamers formulation generally mix with sodium carbonate/dipotassium hydrogenphosphate (Jones *et al*., 1977).

6. Sensory evaluation and multivariate approach

6.1 Descriptive analysis

Descriptive sensory analysis is one of the most comprehensive and informative tools used in sensory analysis. These techniques can provide complete sensory descriptions of products, determine how ingredient or process changes affect characteristics and identify key sensory attributes that promote product acceptance (Lawless and Heymann, 1999; Murray *et al.*, 2001).

In the quantitative descriptive analysis (QDA), various product evaluations are suggested to finalize by trained panelists in making relative judgments

with a high degree of precision (MuÑOz and Civille, 1998). During training, the products are served as illustrative stimuli for the consensus language development. The panes leader works as a communication facilitator without involvement and interference with panel discussions. References can be used for generating sensory terminologies, especially when panelists are confused and disagree with each other on some sensory attributes during training sessions (Stone and Sidel, 2004). The scale direction goes from left to right with increasing intensities (e.g., weak to strong, little to much), this line scale is designed as 6–inch in length with sensory intensities. During data collection, panelist measure sensory intensities (e.g., appearance, ordor, aroma, flavor, texture and aftertaste of product) independently at individual booth without reference served as intensities standard. Panelists are allowed to use different parts of the scaled to determine the sensory intensities by themselves (Lawless and Heymann, 1999; Stone and Sidel, 2004).

The results from QDA are informative for statistical practices to the goal of project. Product difference can be examined by means of one–way ANOVA based on attributes. Statistical procedures such as multivariate analysis of variance, principal component analysis, factor analysis, cluster analysis can be widely applied to QDA data set, means of attributes in the same sensory category can be presented as a graph (Stone and Sidel, 2004).

6.2 Preference test and Just about right

Preference or affective tests are used to assess consumer response to products. They are concerned with acceptability of the product or whether one product is preferred over another. There are two types of preference tests, the paired preference and the 9–point hedonic scale. The 9–point hedonic scale is more useful because it provides a measure of liking for each product, the magnitude of the difference in liking among the products and enables use of parametric statistics such as the analysis of variance to identify significant product differences. Overall it is a more efficient methodology enabling one to test multiple products versus multiple paired comparisons. However, if one wants to know which of two products are

preferred, then the paired comparison is more appropriate. (Stone and Sidel, 2004; Sidel and Stone, 2006).

Just–about–right (JAR) rating scale has been included in the questionnaire in sensory consumer test and marketing research (Gacula *et al*., 2007). These scales, categorical variables, are an approach to the measurement of the perceived attribute intensities that assess whether there is too little, too much, or a JAR level of a particular attribute (Lawless and Heymann, 1999; Gacula *et al*., 2007). It is usually expressed as the percentage of respondents who consider the product according to those scales. The JAR method can also be used to quantify on a non–linear scale which used for quantitative and qualitative analysis of the typicality concept (Cadot *et al*., 2010).

Mean drop analysis is a method for determining it the rating of JAR of respondents for a specific attribute are associated with a drop in choice measure, most commonly overall liking. Mean drop are calculated by subtracting the mean hedonic score for the JAR group from either of the means of the too weak (not enough) or the too strong (too much) group (Cavitt *et al*., 2005). Mean drop is an effective tool for linking attribute performance to overall liking (Schraidt, 2009).

6.3 Multivariate analysis

When a concise understanding on large amounts of data is needed, multivariate statistics can be applied to find the underlying relationships among large numbers of products with many sensory characteristics. Whether it be: cluster analysis, factor analysis, principal component analysis or another multivariate technique etc. Generally, there are often significant correlations between variables. The presence of such covariation means that the significant information can be described by means of a smaller number of variables than those actually measured.

6.3.1 Principal component analysis (PCA)

PCA is a frequently applied method for multivariate overview analysis of sensory data. The main purpose is to interpret any latent factors spanned by many characteristics such as flavor, odor, appearance and texture as well as to find products that are similar or different, and what discriminate those profiles (Westad *et al*., 2003). The data is arranged into a matrix X with one column for each variable and one row for each object. The PCs are orthogonal and describe independent variation structures in the data. The first PC always explains the greatest part, and the following PCs successively explain smaller parts of the original variance. Graphical overviews of the samples and variables are obtained by score– and loading–plots, respectively, ideally showing a large part of the variance in two dimensions. The score plots shows how the samples relate to each other. The samples with similar values of many characteristics will appear close to each other. The pattern of variation is visualized by loading plot. Variables which appear close together in loading plot are positively correlated, whereas those explained by different PCs vary independently. Both sample and loading plots can be interpreted together for better explanation.

6.3.2 Factor analysis

Factor analysis is one of the statistic techniques to identify underlying variables, or factor, that explain the pattern of correlations within a set of observed variables. This technique often used in data reduction to identify a small number of factors that explain most of the variance observed in a much larger number of manifest variables. Factor analysis can be also used to generate hypotheses regarding causal mechanisms or to screen variables for subsequent analysis.

The variable should be quantitative at the interval or ratio level. The category data (such as religion or country of origin) are not suitable for factor analysis. Data for which Pearson correlation coefficients can sensibly be calculated should be suitable for factor analysis.

MATERIALS AND METHODS

Materials

1. Raw material

1.1 Rice bran

There are two types of Thai rice bran; Khao–dawk–mali 105 rice bran was obtained from Royal Chitralada Agricultural project (Bangkok, Thailand) and mixed commercial rice bran was purchased from Patum Rice Mill and Grannary Public Company Limited (PRG, Bangkok, Thailand) during June–August 2009.

1.2 Biopolymers and food grade ingredients

1.2.1 Whey protein isolate (WPI, 97.7 wt% protein) was donated by Davisco Foods International (Le Sueur, MN, USA).

1.2.2 Gum arabic (GA) was donated by TIC Gums (Philadelphia, USA).

1.2.3 Modified starch (MS, named PURITY GUM^{TM} Ultra) was donated by the National Starch LLC (Bridgewater, NJ, USA). This modified starch is a newly developed OSA–modified food starch that is claimed to have advantages over conventional modified starches.

1.2.4 Rice bran oil (RBO) was purchased from local market (Thai Edible Oil Co., Ltd, Bangkok, Thailand).

1.2.5 Maltodextrin (DE18) was purchased from Berli Jucker Company (Bangkok, Thailand).

2. Reagents

2.1 Oil extraction and emulsion formation

2.1.1 Hexane commercial grade (Zen point, Thai oil CO., LTD, Thailand)

2.1.2 Hydrochloric acid, alcoholic potassium hydroxide, ethyl alcohol, potassium hydroxide, isopropyl alcohol, toluene, acetone, potassium hydrogen phthalate, potassium iodine, wijs solution, cyclohexane, glacial acetic, potassium dichromate, sodium thiosulfate, acetic acid, trimethyl pentane, potassium iodine, potassium dichromate, sodium thiosulfate, petroleum ether, sodium hydroxide, phenolphthanein, isooctane, 2–propanl, methanol, 1–butanol, ammonium thiocyanate, barium chloride, cumene hydroperoxide, 3–hexanal, acetone and starch were analytical grade and purchased from Merck (Merck, Germany) and Fluka (Fluka, Switzerland).

2.1.3 Sodium chloride, sodium citrate, sodium hydroxide, barium chloride, ferrous sulfate, ethylenediaminetetraacetic acid (EDTA) and sodium azide were purchased from Sigma Chemical Company (St. Louis, Mo., USA).

2.1.4 Double–distilled water was used to prepare all solutions which was obtained from a water purification system (ELGA purelab option 15 BP, UK).

2.2 Dried powder and the characterization

All of reagent are analytical grade: Lithuim chloride anhydrous (LiCl), potassium acetate (CH_3COOK), magnesium chloride ($MgCl_2.6H_2O$), potassium carbonate anhydrous (K_2CO_3), sodium bromide (NaBr), cupric chloride ($CuCl_2.2H_2O$), sodium chloride (NaCl) and potassium chloride (KCl) were purchased from Univar (Ajax finechem., Ltd., Australia).

3. Equipments

3.1 Oil extraction and the qualities

3.1.1 Sieving machine (80 mesh) (SC–R–1, KluayNamThai, Thailand)

3.1.2 Autoclave (88L4, Scientific promotion Co., LTD)

3.1.3 Soxhlet apparatus with hot plate (BI Barnstead, Electrothermal Enginerring, UK) and cooler (CTL 911)

3.1.4 Vacuum packing (Food sarver® Vacloc® vacuum packaging, Jarden Cooperation, Korea)

3.1.5 Rotary evaporator (R152, BÜCHI, Switzerland) with pump (B–160, Vacobox) and recirculation chiller (BÜCHI700)

3.1.6 Cold press extractor (Feed in by D.C motor model: z50/20 50 watts, 135 rpm, press by 1 phase induction motor with750 watts, 50Hz, RPM1420) and extracted by high pressure with cylinder screw type 20–25 rpm (CE. Chang Pang Co.Ltd., Taiwan)

3.1.7 Aspiratory pump (A–3S, EYEKA)

3.1.8 Refrigerated centrifuge (model H–80R, Kokusan, Japan)

3.2 Emulsion formation and encapsulation

3.2.1 High speed blender (Biospec Products Inc., Bartlesville, USA)

3.2.2 High speed homogenizer (model L4RT, Silverson, UK)

3.2.3 Hand homogenizer (model Ultra–Turrax T25 basic, IKA Co Ltd., Germany)

3.2.4 High pressure homogenizer (model 101, Microfluidics, Newton, Massachusetts, USA)

3.2.5 High pressure homogenizer (model 15MR–8TBA, APV, USA)

3.2.6 Spray dryer (Mobile, Minor Spray Dryer, Niro A/S, Soeborg, Denmark)

3.2.7 Hot plate stirrer (model Ms–H–Pro, Dragon lab, USA)

3.2.8 pH meter (model 510, Eutech instruments, UK)

3.2.9 Water bath (WB22, Memmert, Germany) with shaker (SV 1422, Germany)

3.3 Analytical equipments

3.3.1 Analysis of crude rice bran oil

3.3.1.1 Hot air oven (FD115, Binder, USA)

3.3.1.2 Vacuum oven (VD53, Binder, USA)

3.3.1.3 Tintometer (model PFX990, Lovivond, Germany)

3.3.1.4 Refractometer (model RX–5000α, ATAGO, Japan)

3.3.1.5 Density meter (DA–100M, Mettler Toledo, Japan)

3.3.1.6 Saponification unit (Appendix B)

3.3.1.7 Unsaponification unit (Appendix B)

3.3.1.8 UV–visible spectrophotometer (model UV–160A, Shimadzu, Japan)

3.3.2 Analysis of rice bran oil in water emulsion

3.3.2.1 Laser light scattering instrument (Mastersizer2000, Malvern Instruments, Worcestershire, UK)

3.3.2.2 Dynamic light scattering instrument (Zetasizer Nano series –Zen 3600, Malvern Instruments, Worcestershire, UK)

3.3.2.3 Spectrophotometer (Genesys 20, Thermo Spectronic, Waltham, MA)

3.3.2.4 Shimadzu GC–2014A gas chromatograph (GC) equipped with an AOC–5000 auto–injector (Shimadzu, Tokyo, Japan) with A 50/30 μm divinylbenzene / carboxen / polydimethylsiloxane (DVB/carboxen/PDMS) stable flex SPME fiber (Supelco, Bellefonte, PA)

3.3.2.5 A SPME fiber with a fused–silica capillary column (30 m × 0.32 mm i.d. × 1 μm) coated with 100% polydimethylsiloxane (Equity–1, Supelco)

3.3.3 Analysis of dried powder

3.3.3.1 Hot air oven (FD115, Binder, USA)

3.3.3.2 Novasina (a_w TH200, Novasina, Pfaffikon, Switzerland)

3.3.3.3 Table top centrifuge (model DSC–200A–2, Digisystem Laboratory Instrument Inc, Taiwan)

3.3.3.4 Spectrophotometer (CM–3500d, Minolta Co., Ltd, Osaka, Japan)

3.3.3.5 Particle size analyzer (Mastersizer S version 2.19, Malvern Instruments Ltd., Worcestshire, UK)

3.3.3.6 Density tester (ASTM version, Varian, USA)

3.3.3.7 Differential scanning calorimetry (Mettler Toledo 822e/ liquid N_2 cooling, Schwerzenbach, Switzerland)

3.3.3.8 Coating machine (Eiko Engineering 1B2, Nagoya, Japan).

3.3.3.9 Scanning electron microscopy (JSM–5600LV, JEOL Ltd., Tokyo, Japan)

3.3.3.10 UV–visible spectrophotometer (model UV–160A, Shimadzu Japan)

Methods

1. Oil extraction and oil qualities measurement

1.1 The preparation of rice bran

The rice bran are from two types of Thai rice: Khao–dawk–mali 105 (*Oryza sativa L. cv.*) and mixed rice bran. Khao–dawk–mali 105 was obtained from Royal Chitralada Agricultural project (Bangkok, Thailand) while mixed rice bran was purchased from Patum Rice Mill and Grannary Public Company Limited (PRG, Bangkok, Thailand). The bran was passed though sieve (80 mesh), followed by pre–treatment under 100 ºC for 10 min by autoclave. They were then packed in plastic bags made from linear low density polyethylene (LLDPE), placed in containers and stored under –17 ± 2 ºC before the experiment was carried out (Appendix A).

1.2 Oil extraction methods

Rice bran was kept at –17 ºC. The bran was equilibrated to 4 ºC for 12 h and then it was equilibrated to room temperature before use. Crude oil was extracted using the methods described below:

1.2.1 Solvent extraction

For the solvent extraction, the extraction procedure was modified from semi–continuous extraction process. Rice bran (500 g) was extracted with hexane (4 L) using soxhlet apparatus under the heat at 65 ºC for 8 h. The solvent was then removed from the extracts by rotary evaporation under vacuum (200–335 mbar, 60–75 ºC) to obtain the concrete contents (Appendix Figure–Table B1). After cooling, the oil was centrifuged for 15 minutes in 7,000 rpm using the refrigerator centrifuge (model H–80R, Kokusan, Japan).

1.2.2 Cold pressed extraction

The modified extraction procedure for the cold pressure from Tsaknis *et al*. (1998) was performed. Rice bran was first fed from the sample feeding on top of the cold presses machine (z50/2 50 watts, 135 rpm), then the bran was extracted by high pressure with cylinder screw type 20–25 rpm (CE. Chang Pang Co.Ltd., Taiwan). The temperature of bran residual was controlled to be below 67 °C to prevent the degradation of active compounds in oil. Crude oil was passed through filter paper no.1, kept in brown bottle and stored under 4 ± 1 ºC. After cooling, the oil was centrifuged for 15 minutes in 7,000 rpm using the refrigerator centrifuge (model H–80R, Kokusan, Japan).

1.3 Physicochemical characterization

Oil extracted characterization was performed according to the Thai Industrial Standards Institute (TISI, 2516) and the Official method of american oil chemist's society (AOCS, 1998). The analyzed parameters were: water and volatile matter (AOCS Ca 2d–25), color (AOCS Cc 13e–92), refractive index (AOCS Cc 7–25), saponification number (AOCS Cd 3–25), unsaponifiable matter (AOCS 6b–53), acid value (AOCS Cd 3d–63), iodine value (AOCS Cd 1d–92), peroxide value (AOCS Cd 8b–90) and specific gravity (TISI 44–2516).

Fatty acid composition of the oil was determined after methylation by Gas Liquid Chromatography (Jham *et al*., 1982; AOCS 1997). Tocopherol and tocotrienol were determined using High Performance Liquid Chromatography (AOCS Ce 8–89). Phytosterol content was measured according to a method described by Laakso (2005).

1.4 Statistical analysis

Each result was expressed as the mean ± S.D. of three determinations. The data were performed using Microsoft Excel 2010.

2. The formation and stability of rice bran oil–in–water emulsion

In Thailand, there is large rice consumption and a large amount of rice exported every years. As a result, there is also a lot of rice bran which is the by product from rice milling process. Rice bran can be extracted to obtain rice bran oil, which can then use in a wide variety of food products. In order to wider the application of rice bran oil for the food industry, the effect of the environment stresses on the rice bran oil–in–water emulsion stability and oxidation stability during the product storage should be explored.

One of the objectives of this study is therefore to investigate whether rice bran oil can be used to form stable oil–in–water emulsions, which are suitable for the application in various food products. The commercial rice bran oil (Thai edible Oil Co., Ltd, Bangkok, Thailand) was selected to use as the disperse phase in emulsion system. The influence of pH, ionic strength and heating on the stability of the biopolymer–stabilized rice bran oil droplets were examined.

2.1 Preparation of oil–in–water emulsions

Aqueous phases were prepared by dispersing WPI (0.02–5.0%), GA (0.05%–10.0%) or MS (0.2–5.0%) in aqueous buffer solutions (10.0 mM sodium citrate, 0.01 wt% sodium azide, pH 7.0) followed by stirring at room temperature overnight to ensure complete dispersion and hydration. Rice bran oil–in–water emulsions were prepared by homogenizing 5.0 wt% oil phase with 95.0 wt% aqueous phase at ambient temperature. An emulsion pre–mix was prepared using a high–speed blender (2 minutes, Biospec Products Inc., Bartlesville, USA), which was then passed through a high pressure homogenizer (model 101, Microfluidics, Newton, Massachusetts, USA) three times at 9,320 psi. (Figure 16).

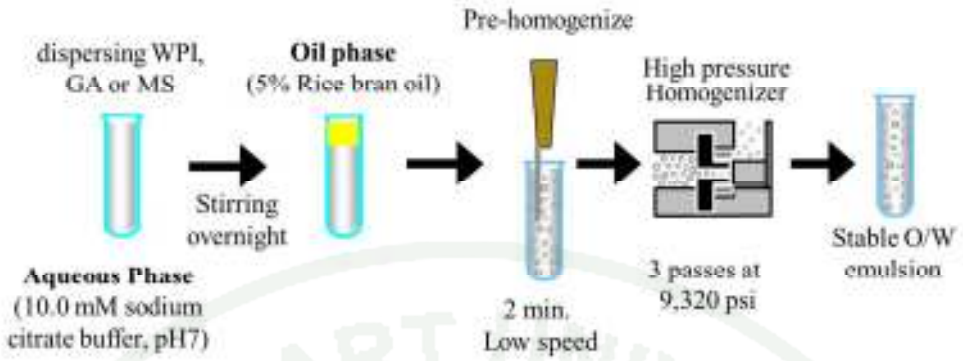


Figure 16 The preparation of emulsions stabilized with different biopolymer.

2.2 The effect of biopolymer type and enviroment stress on rice bran oil emulsion stability

2.2.1 Emulsion preparation

Aqueous phases were prepared by dispersing 0.45% WPI, 10.0% GA or 1.0% MS in aqueous buffer solutions (10.0 mM sodium citrate, 0.01 wt% sodium azide), followed by stirring at room temperature overnight to ensure that dispersion and hydration were completed. These levels of emulsifiers were selected based on the previous study (in section 2.1) as they were the minimum amounts of the emulsifier required to form relatively small droplets (d_{43} < 0.6 μm) during homogenization. Rice bran oil–in–water emulsions were prepared by homogenizing 5.0 wt% oil phase with 95.0 wt% aqueous phase at ambient temperature. An emulsion pre–mix was prepared using a high–speed blender (2 minutes, Biospec Products Inc., Bartlesville, USA). It was then passed through a high pressure homogenizer (model 101, Microfluidics, Newton, Massachusetts, USA) three times at 9,320 psi.

2.2.2. The influence of environment stresses on emulsion stability

The stability of the various emulsions to pH, ionic strength and temperature was tested. Three 5 wt% oil–in–water emulsions were prepared using different emulsifier types. The concentrations determined in preliminary experiments

were described previously in Section 2.1: WPI (0.45 wt%); GA (10.0 wt%) and MS (1.0 wt%), respectively.

2.2.2.1 Stability to pH

Emulsion samples were prepared in aqueous buffer solutions, then the pH was adjusted to the desired final value (pH 3 to 8) using either NaOH and/or HCl solution. Emulsion samples (10 mL) were then transferred into glass test tubes (160 × 15 mm) and stored at ambient temperature overnight prior to analysis.

2.2.2.2 Stability to ionic strength

Emulsions (pH 7) were diluted with different amounts of NaCl and buffer solution to form a series of samples with the same droplet concentration in oil: salt solution ratio of 1:4, but different salt concentrations (0 to 500 mM NaCl). The emulsions were stirred for 30 minutes and transferred into glass test tubes (160 × 15 mm) and stored at ambient temperature overnight prior to analysis.

2.2.2.3 Stability to heating

Emulsions (pH 7) were prepared containing either 0 or 150 mM NaCl and 10 mL samples were transferred into glass test tubes (160× 15 mm), which were stored in a water bath for 30 minutes at a fixed temperature ranging from 30 to 90 °C. The emulsion samples were then immediately placed at room temperature and stored overnight prior to analysis.

2.3 The effect of biopolymer type at different pH (3,7) on oxidation stability of rice bran oil emulsion

Since, in the previously experiment, rice bran oil in water emulsions were prepared using similar concentrations (2.2.1). The further study required was to compare the oxidative stability of lipid droplets stabilized by different biopolymer emulsifiers at pH 3 and 7.

Aqueous phases were prepared by dispersing 0.45% WPI, 10.0% GA or 1.0% MS in aqueous buffer solutions (10.0 mM sodium citrate, 0.01 wt% sodium azide) with and without 200 μM of ferrous iron–EDTA (Ferrous iron–EDTA solutions were made by dissolving 200 μM EDTA and 200 μM ferrous sulfate in buffer solutions–10 mM sodium citrate, 0.01 wt% sodium azide), followed by stirring at room temperature overnight to ensure that dispersion and hydration were completed. These amount of emulsifiers were selected based on the previous study. Rice bran oil–in–water emulsions were prepared by homogenizing 5.0 wt% oil phase with 95.0 wt% aqueous phase at ambient temperature. An emulsion pre–mix was prepared using a high–speed blender (2 minutes, Biospec Products Inc., Bartlesville, USA). It was then passed through a high pressure homogenizer (Model 101, Microfluidics, Newton, Massachusetts, USA) three times at 9,320 psi. Samples of emulsions were then adjusted to either pH 3.0 or 7.0 using NaOH and/or HCl.

2.4 Emulsion characterization

2.4.1 Measurement of emulsion stability

2.4.1.1 Particle size: the particle size distribution (PSD) of the emulsions was measured using a laser light scattering instrument (Mastersizer2000, Malvern Instruments Ltd., Worcestershire, UK). This instrument measures the intensity of laser light scattered from a dilute emulsion, and then reports the particle size distribution that gives the closest fit between theoretical calculations (Mie theory) and experimental measurements of intensity *versus* scattering angle. To avoid

multiple scattering effects, emulsions were diluted with the same buffer as the continuous phase. Particle size measurements are reported as volume–weighted mean diameters d_{43} (= $\sum n_i d_i^4 / \sum n_i d_i^3$), where n_i is the number of particles with diameter d_i. The refractive indices of the dispersed and continuous phases used in the calculations of the particle size distribution were 1.464 and 1.330, respectively. The imaginary part of the refractive index of the rice bran oil was assumed to be zero although it did have a slight yellowish color, which may therefore have had some effect on the reported particle size distributions. It should be noted that the dilution and stirring are likely to disrupt any weakly flocculated droplets so the particle size data on highly aggregated emulsions should be interpreted with caution.

2.4.1.2 The electrical charge (ζ–potential) of lipid droplets in the emulsions was determined using a particle electrophoresis instrument (ZEN3600, Nano–series, Zetasizer, Malvern Instruments, Worcestershire, UK). Emulsions were diluted until they gave an instrument attenuation factor of ~6 using buffer solution at the same pH and NaCl concentration as the initial sample. The emulsions were agitated prior to analysis to ensure that they were homogeneous. The ζ–potential of each individual sample was calculated from the average of 2 freshly prepared samples with at least 2 replications per sample. The instrument used the Smoluchowski approximation to calculate the ζ–potential from the measured electrophoretic mobility of the particles.

2.4.1.3 Creaming index: emulsion samples (10 mL) were placed in glass test tubes (16× 150 mm) and then stored at ambient temperature for 7 days before analysis. The susceptibility of the emulsions for creaming was ascertained by measuring the height of the boundary layer between the opaque droplet–rich layer at the top and the transparent or turbid droplet–depleted layer at the bottom of the test tubes. Creaming results were reported as the Creaming Index = 100 × (height of interface)/(height of total emulsion) (Demetriades and McClements, 2000).

2.4.2 Lipid oxidation measurements

Samples (1 mL) were placed in 10 mL glass vials, sealed with polytetrafluoroethylene (PTFE)/butyl rubber septa using a crimper and aluminum seals, and incubated at 37 °C in the dark. Oxidation was followed by measuring hydroperoxide (primary product) and headspace hexanal (secondary product) formation.

Lipid hydroperoxides were determined using a method adapted from Mancuso *et al*. (1999). Emulsion samples (0.3 mL) were added to 1.5 mL of isooctane/2–propanl (3:1 v/v). The mixture was then vortexed (10 sec, 3 times) and the organic solvent phase was isolated by centrifugation at 1000g for 3 minutes. The organic solvent phase (0.2 mL) was added to 2.8 mL of methanol/1–butanol (2:1 v/v), followed by 15 μl of 3.9 M ammonium thiocyanate, and 15 μl of ferrous iron solution (prepared by adding equal amounts of 0.132 M $BaCl_2$ and 0.1444 M $FeSO_4$). After 20 minutes, the absorbance was measured at 510 nm using a spectrophotometer (Genesys 20, Thermo Spectronic, Waltham, MA). Lipid hydroperoxide concentrations were determined, using a standard curve made from cumene hydroperoxide.

Headspace hexanal was determined according to a method described by Panya *et al*. (2010) using a Shimadzu GC–2014A gas chromatograph (GC) equipped with an AOC–5000 auto–injector (Shimadzu, Tokyo, Japan). A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/carboxen/PDMS) stable flex SPME fiber (Supelco, Bellefonte, PA) was inserted through the septum into the vial and exposed to the sample headspace for 15 minutes at 55 °C extraction time 2 minutes. The SPME fiber was desorbed at 250 °C for 3 minutes in the GC detector at a split ratio of 1:5. The chromatographic separation of volatile aldehydes was performed on a fused–silica capillary column (30 m × 0.32 mm i.d. × 1 μm) coated with 100% poly(dimethylsiloxane) (Equity–1, Supelco). The temperatures of the oven, injector, and flame ionization detector were 65, 250 and 250 °C, respectively. Sample run time was 10 minutes. Relative hexanal concentrations were determined

using a standard curve made from dissolving different amounts of 3–hexanal in 10 mL of methanol and the dispersing them in buffer solution. In emulsions, some of the hexanal may partition into the oil phase so the reported head space hexanal concentrations should only be used to compare the relative formation of secondary products between different emulsion samples.

2.4.3 Statistic analysis

All of the experiments were done in triplicate. Differences between means were determined with the least significant difference at $P \leq 0.05$.

3. The encapsulation of rice bran oil and physical properties of powder

Spray drying was selected to use for encapsulated crude rice bran oil in order to reduce the deterioration of bioactive compounds from the environmental factors (such as temperature, light, moisture and oxygen). The crude rice bran oil (Khao–dawk–mali 105) under cold pressed extraction was use to examine the influence of wall material composition on the formation, stability and physicochemical properties of encapsulated rice bran oil.

3.1 The preparation of encapsulated rice bran oil powder (10% Oil)

Aqueous phases were prepared by dispersing WPI (0.02%–10.0%), or MS (0.2–10.0%) in aqueous buffer solutions (10.0 mM sodium citrate, pH 7.0), followed by stirring at room temperature overnight to ensure that dispersion and hydration were completed. Rice bran oil–in–water emulsions were prepared by homogenizing 10.0 wt% oil phase with 90.0 wt% aqueous phase at ambient temperature. An emulsion pre–mix was prepared using a hand homogenizer, which was then passed through a high pressure homogenizer (model 15MR–8TBA, APV, USA) at 5500 psi. (the same procedure in Figure 16).

Spray drying process was performed in a spray dryer (Mobile Minor Spray Dryer, Niro A/S, Soeborg, Denmark) by a peristaltic pump. Rice bran oil–in–water emulsions were atomized to small droplets by a centrifugal vaned atomizer wheel with a rotational speed of 20,000 rpm (3 bar air pressure) in a co–current air flow system. The inlet air temperature was set at 180 ± 2 ºC and the outlet air temperature was kept at 90 ± 2 ºC by varying the feed rate in the range of 10–12 mL/min.

3.2 Physical properties of spray–dried powder

3.2.1 Water content and water activity

The water content was measured by drying a powder sample (3 g) in an oven and dry at 102 ± 2 °C for 2 h. After it was cooled to room temperature in desiccator, it was weighed and dried at 102 ± 2 °C for 1 h. The process of dry, cooling and weight were repeated until its weight was constant (do not differ by more than 0.5 mg) (A/S Niro Atomizer, 2003a) The water activity was measured using a Novasina (TH200, Switzerland).

3.2.2 Density

The bulk and tapped densities of powder were calculated from the relationship: mass/volume. The powder was loaded gently into a 100 mL tared graduated cylinder until it is at the 100 mL mark, then the powder was weighed. The volume observed directly from the cylinder was then used to calculate the bulk density (ρ_{bulk}). For the tapped density (ρ_{tapped}), the cylinder was tapped 1,250 times using Vankel Tapped Density Tester (ASTM version, Varian, USA). The volume of the sample was then read off and used in the calculation.

Flowability and cohesiveness of the powder were evaluated in terms of Carr index (*CI*) (Carr, 1965) and Hausner ratio (*HR*) (Hausner, 1967). Both *CI* and *HR* were calculated from the bulk (ρ_{bulk}) and tapped (ρ_{tapped}) densities of the powder as shown below:

$$CI = [(\rho_{tapped} - \rho_{bulk})/ \rho_{tapped}] \times 100 \quad (4)$$

$$HR = \rho_{tapped} / \rho_{bulk} \quad (5)$$

Therefore, the classification of powder flowability based on Carr index and classification of powder cohesiveness based on Hausner ration were presented in Table 8 and 9, respectively.

Table 8 Classification of powder flowability based on Carr index (*CI*).

CI (%)	Flowability
<15	Very good
15–20	Good
20–35	Fair
35–45	Bad
>45	Very bad

Source: Jinapong *et al*. (2008)

Table 9 Classification of powder cohesiveness based on Hausner ratio (*HR*).

HR (%)	Cohesiveness
<1.2	Low
1.2–1.4	Intermediate
>1.4	High

Source: Jinapong *et al*. (2008)

3.2.3 Particle size measurement

For the spray–dried powder, the particle size distribution and volume–weighted mean diameter were measured using a sieve analysis. A vibratory sieve shaker (Retsch GmbJ and Co., Haan, Germany) with a series of seven sieves was used to determine their particle size distribution. The aperture sizes of sieves were 125, 150, 180, 250, 355, 500, 710 and 1000 μm. Spray dried powder (50 g) was put on the sieve's series and shaken at 60 Hz for 30 minutes. The size distribution was described by log–normal distribution relationship. From the log–probability plots, the mass–weighted geometric mean diameter and the geometric standard deviation defined by the slope of the log–normal curve were determined.

3.2.4 Wettability

Wettability of the powder sample was determined according to A/S Niro Atomizer (2003) with some modifications. An amount of distilled water (100 mL) at 25 ± 1 ºC was poured into a 250 mL beaker. A glass funnel held on a ring stand was set over the beaker with the height between the bottom of the funnel and the water surface of 10 cm. A test tube was placed inside the funnel to block the lower opening of the funnel. The powder sample (1 g) was placed around the test tube. The tube was then lifted while the stop watch was started at the same time. Finally, the time for the powder to become completely wet was recorded (visually assessed as when all the powder particles were penetrated the surface of the water).

3.2.5 Dispersibility

Dispersibility measurement was performed according to the procedure described in A/S Niro Atomizer (2003) with some modifications. Distilled water (10 mL) at 25 ± 1 ºC, was poured into a 50 mL beaker. The powder (1 g) was added into the beaker. The stop watch was started and the sample was stirred vigorously with a spoon for 15 s, making 25 complete movements back and forth across the whole diameter of the beaker. The reconstituted creamer was poured

through a sieve (212 μm). The sieved creamer (1 mL) was transferred to a weighed and dried aluminum pan and dried for 4 h in a hot air oven at 105 ± 1 ºC. The dispersibility of the powder was calculated as follows:

$$\% \text{ dispersibility} = \frac{(10+a) \times \%TS}{a \times (100-b)/100} \quad (6)$$

where a is amount of powder (g) being used, b is moisture content in the powder, and % TS is dry matter in percentage in the reconstituted creamer after it has been passed through the sieve.

3.2.6 Total and free oil of spray–dried powder

The total oil and free oil contents of the powder were determined according to the A/S Niro Atomizer (2003). This is a standard method which may be used for all milk powder and other dried dairy products. The 1.5 g of powder was weighted and loaded into the shaking cylinder. Then, 10 mL of water and 1.5 mL of NH_3–solution were added to mixture. After that the mixture was shaked occasionally and heated in a water bath for 15 minutes to 65 ºC.

Ethyl ether (25 mL) was added into the solution, which was mixed by turning the cylinder up and down for 1 minute. The 25 mL of petroleum ether was then added and the mixture was allowed to stand for at least 2 h. After that, the ether phase was transferred to an erlenmeyer flask. This total oil extract was repeated for 2 times. An erlenmeyer flask was dried at 102 ± 2 ºC until constant weight was reached and the total fat was calculated using the equation shown below.

For free fat content, 5 g of the powder was weighted and loaded into 250 mL erlenmeyer flask. The petroleum ether (50 mL) was added to the mixture. The mixture was then shaked by the shaking device for 15 minutes. After the solution was filtered, the filtrate was collected. The 25 mL of the filtrate was transferred into the Erlenmeyer flask and dried in the drying oven for 1 h at (105 ± 2 ºC). The, it was

cooled in the desiccator and weighed. The % of free fat was calculated using the equation shown below.

$$\% \text{ Total fat} = \frac{W_1 \times 100}{W_2} \quad (7)$$

$$\% \text{ Free fat} = \frac{a \times 25 \times 2 \times 100}{25 - \frac{a}{0.91} \times b} \quad (8)$$

where, W_1 is weight in g of the evaporation residue, W_2 is weight in g of the powder used, a is evaporation residue from 25 mL of solvent, b is g of powder used.

Encapsulation efficiency (EE) was calculated as follows:

$$\% \text{ EE} = \left[\frac{\text{Total oil-Free oil}}{\text{Total oil}}\right] \times 100 \quad (9)$$

3.2.7 Phytosterol content in powder

Phytosterols content was determined according to a method described by Laakso (2005). A method was based on hot saponification of a sample with ethanolic potassium hydroxide in the presence of an internal standard (5α–cholestanel). Acid hydrolysis was used to release matrix–incorporated bound sterols or sterols from steryl glycosides before the saponification step. After saponification, the unsaponifiable material containing phytosterols is extracted into and organic solvent, followed by the evaporation of the solvent to dryness. Sterols are separated as trimethysilyl ether (TMS) derivatives of phytostanols and phytosterols trimethylsilyl ether derivatives.

Gas–liquid chromatograph (GC) on a column detected with a flame ionization detector (FID) (25 m x 0.25 mm i.d., film thickness 0.25 μm) was used 1.0 μL of derivatized sample solution was injected into the column split injection

(270 ºC, slit ratio 1:10). The components were separated isothermally at 300 ºC and detected with the FID (320 ºC). The carrier gas was Helium. Phytosterols standard (mixed phytosterol) was purchased from Sigma (St. Louis, MO, USA) to confirm peak identifications.

3.2.8 Scanning electron microscopy

The morphology of dried powder was examined by scanning electron microscopy. Samples were mounted on the aluminum specimen holder with double–sided tape. The specimen holder was loaded in coater (Eiko engineering 1B2, Nagoya, Japan). The sample was coated with gold palladium and viewed under scanning electron microscopy (JSM–5600LV, JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 10 kV.

4. Physicochemical properties of encapsulated rice bran oil

Spray–dried rice bran oil powder was placed in aluminum foil and stored under frozen temperature until further analyses. For storage studies, spray–dried oil powder (1 g each) were place in proximity equilibration cell (PEC) with different relative humidity (RH) of 11–87%RH, prepared form saturated salt solution. The 15 g of spray–dried powder were also placed in aluminum foil bag and the bags were incubated at 25, 35 and 45 ºC. For studies on the effect of storage temperature, some samples were withdrawn at frequent time intervals for analyses.

4.1 The study of sorption isotherm of powder

To determine the isotherm of dried powder stabilized by whether WPI or MS, triplicate 1 g were equilibrated against the saturated salt solutions (11% LiCl, 23% CH_3COOK, 33% $MgCl_2$, 43% K_2CO_3, 57% NaBr, 67% $CuCl_2$, 75% NaCl and 86% KCl) as a modified PEC described by Lang *et al*. (1981). The sample holder was a plastic weighing dish with 45 mm diameter and 55 mm deep. A clean dry sample holder was weighed and placed in the PEC containing saturated salt solution for 24 h

at 25 ± 2 ºC. The sample holder was weighed again after 24 h and 1 g sample was placed in the holder in an approximately 2 mm thick layer. The sample holder was then inserted in the PEC for equilibration. The required time for equilibration was 1–2 weeks, based on the change in sample weight, which did not exceed 0.1%.

Sorption isotherms are generally described by mathematical models based on empirical or theoretical criteria, which can be easily found in the literature. Thus, in this work, sorption isotherms data were modeled according to GAB and BET models, using the Solver algorithm of Microsoft Excel. The goodness of fit was evaluated by the determination coefficient (R^2) and the mean relative deviation modulus (E), where V_E is experimental value and V_P is predicted value.

$$E = \frac{100}{N} \sum_{i=1}^{N} \frac{[V_E - V_P]}{V_E} \qquad (10)$$

Glass transition temperature (T_g) were measured from Differential Scanning Calorimetry (Mettler Toledo 822e with liquid N_2 cooling). The diagrams were analyzed using STAR^e thermal analysis software, version 8.1 (Mettler Toledo Schwerzenbach, Switzerland). Samples of spray–dried powder (10 mg) were transferred to DSC aluminum pans (40 μL) and equilibrated in saturated salt solution (11%–43%). The equilibrated samples in the DSC pans were hermitically sealed. Duplicated samples of each powder were analyzed. An empty pan was used as a reference. The samples were scanned first to 110 ºC above the predetermined T_g (onset) at 5 ºC/min, then cooled at 5 ºC/min to 0 ºC below T_g. The second heating scan 5 ºC/min was run to 200 ºC above the glass transition temperature range. These thermal properties were modified according to a method described by Silalai and Roos (2010).

4.2 Kinetic analysis of lipid degradation

4.2.1 Lipid oxidation measurements

Lipid hydroperoxides were determined using a method adapted from Klinkesorn *et al*. (2005) which was the same procedure described in section 2.4.2.

4.2.2 Kinetic reaction order

The lipid peroxidation of dried powder was changed during storage, increased with time, temperature and humidity. The data was best fit by zero or first order as equation below;

$$\text{Zero order:} \quad C = C_0 + kt \qquad (11)$$

$$\text{First order:} \quad \ln(C) = \ln C_0 - kt \qquad (12)$$

$$\text{Second order} \quad \frac{1}{C} = \frac{1}{C_0} + kt \qquad (13)$$

where C_0 is the initial peroxide content at day 0 after spray drying and C is the peroxide content after t (time) of stability treatment at a given temperature. Degradation rate constants (k) were obtained from the slope of a plot of Y versus time (x).

4.3 Changes in visual color

The colors of the spray–dried powder were measured in the term of the CIE L*, a* and b* using spectrophotometer (CM–3500d, Minolta Co., Ltd, Osaka, Japan).

5. Application of rice bran oil powder in food model

The necessary thing of the product development is to understand consumer's behavior on the products as it results in the consumer decision in buying products and the success in products launch. Therefore, the study in this section aims to understand the consumer needs on the rice bran oil product based on the consumer survey about the product samples.

5.1 Data collection and consumer survey

This research has been distributed into two major sections including primary data and secondary data. The questionnaire set consists of three parts. The first part is the demographic profile, while the second part is the behavior and the needs of the respondents toward rice bran and rice bran oil products. The final part is about factors influencing on the consumer buying decisions for rice bran and rice bran oil products. A buying decision score was rated using the five–point likert scale. For the questionnaire set, the questionnaire was used to test the reliability of the questions (n=20) first by Cronbach's Alpha coefficient method ($P \leq 0.05$), then the questionnaires were adjusted.

The data were then collected from the questionnaire and the interview of 213 consumers. The sampling method used by the researcher was judgment sampling method. The survey was done by 213 consumers who live in Bangkok and its vicinity during January 2008 to March 2008. The statistical analysis included: frequency, percentage, arithmetic mean, mode, standard deviation, and chi–square test. Factor validity was assessed by factor analysis of the buying decision scale items using principal component analysis and oblique rotation by the varimax approach.

5.2 Rice bran oil powder application in food model

In order to apply the rice bran oil powder in the food model, rice bran oil powder was used as coffee creamer. This food model was recommended by consumer

based on consumer survey information. The determination of sensory characteristic of coffee creamer using a generic sensory descriptive analysis (GDA) and just about right (JAR) of powder in food model showed as below:

This work was divided into two parts. The first part was an analysis of descriptive to identify the sensory attributes of coffee creamer products (4 samples from commercial product and 2 samples from rice bran oil powder; Table 10) using the 9 trained panels in order to identified product characteristics. The multivariate technique was used for describing the relation between the physical properties and sensorial properties of the products by principal component analysis (PCA).

The second part, in order to satisfy consumer demands for coffee creamer products, it required not only successful product development but also understanding of factor underlying consumer perception and acceptance by evaluated JAR and reported the percentage of acceptability.

5.2.1 Sample preparation

Sample (2 g) was dissolved with hot water (100 mL) and the mixture was stirred until homogenous. All 6 samples were hold the temperature 50 ºC under water bath. Ten mL of sample solution were transferred to plastic cups covered with plastic lids. Each of samples was served individually to panelists at 50 ºC, and evaluated immediately after serving. Purified water and white bread were used by the panelist for palate cleaning between samples.

5.2.2 Sensory descriptive analysis

A generic sensory descriptive analysis (Lawless and Heymann, 1999) was used to develop the lexicon and methodology for the evaluation of coffee creamer. A panel of nine trained panelists (24–34 years old) was from Kasetsart University. This panel had experience in descriptive analysis for at least 2–3 years. Each panelist was completed 3–6 sessions (3 h each). Overall, the training session

includes concept alignment and agreement, lexicon development (term and their definitions, references and use of scale), sample handling, practicing and product evaluation (quantifying the intensity of attributes). A total of 15–16 attributes (Table 10) were defined and agreed by the panelists and were evaluated using a continuous 15–cm line scale, ranging from no intensity to extremely strong. All 6 coffee creamers were independently evaluated in duplicated with 3 samples evaluated per session. Each sample was labeled with a 3–digit random number and the order of sample presentation was randomized to avoid bias. Before evaluation the next samples, the panelists were required to rinse their palate using water and white bread for palate cleaning.

Table 10 List of coffee creamer products used for descriptive analysis.

Bands	Code	Oil type	% Total fat	Casien/sodium casinate	Syrup/ Maltodextrin
Coffee mate	A	Hydrogenate oil	34	–	61
Year mate	B	Palm oil	32	–	50
Khao shong	C	Palm oil	34	2	58
Buddy dean	D	Soy oil	38.0	–	50
WPI–Powder	E	Rice bran oil	32.7	3.5	50
MS–Powder	F	Rice bran oil	30.3	7.0	55

All sensory descriptive and physical/sensorial properties were subjected to ANOVA (analysis of variance) and MANOVA (multivariate analysis of variance) at $P \leq 0.05$ using SPSS 12.0 (SPSS Inc., Thailand). MANOVA was performed to determine if all samples were different when all sensory attributes and/or physical attributes were simultaneously considered. Duncan's multiple range test (DMRT) was performed to locate differences among samples. The relationship between sensory descriptive and physical properties was determined using Pearson correlation coefficients (r). Mean score of each attribute (only those that were significant by ANOVA) were used in PCA and a product attribute biplot from PC1 and PC2 was created by the XLstat2011 software (Addinsoft, Paris).

5.3 Consumer preference and just about right

The coffee sample contained 1.7 g of commercial coffee powder, 10 g of coffee mate (rice bran oil powder), 7 g of sugar and 100 mL of hot water with temperature of no less than 80 ºC.

5.3.1 Coffee solution preparation

Two g of commercial coffee powder (Nescafe red cup, Pathumthanee) was dissolved in hot water (100 mL) and stirred until homogenous. Ten g of coffee creamer and 7 g of sugar were then added, respectively. Hot coffee sample was kept at the temperature of 70–80 ºC. Approximately 15 mL of coffee sample was transferred to a cup glass. Three samples were served to panelists and evaluated immediately after serving. Fresh water was used by the panelists for palate cleaning between samples.

5.3.2 Consumer preference

The consumer preference on rice bran oil powder in a food product prototype was investigated. The central location test (CLT) was conducted with 122 consumers at the canteen in Kasetsart University. Each consumer was received 3 samples of the powder (2 samples from rice bran oil powder and once from commercial product) and evaluated 3 samples of coffee solution with the creamer. The product characteristics such as color, caking, hand feel, solubility, opaque, rice bran oil odor, rice cooker flavor, mouth coating etc. The overall liking were determined using the 9–point hedonic scale (1 –dislike extremely, 5 – neither like nor dislike and 9 –like extremely). The just about right scale (1 = not enough, 2 = just about right, JAR and 3 = too much) was also used to evaluate color, caking, rice bran oil odor (RBO_Od), milk flavor (milk flavor) and mouth coating. In addition, the just about right for the products was explored using Binomial test.

6. Place and duration

6.1 Department of Product Development, Faculty of Agro–Industry, Kasetsart University, Thailand

6.2 Department of Food Science, University of Massachusetts Amherst, Amherst, MA, USA

All experiments were carried out from July 2008 to September 2011.

RESULTS AND DISCUSSION

1. Rice bran oil qualities and bioactive compounds

The primary preparation of rice bran was conducted to get rid of the impurity. After collecting rice bran from the factories, it was then packed in LLDPE bag and kept under the temperature of –18 ºC. Before extracting the rice bran oil, the frozen rice bran was thawed at 4 ºC for 1 day. The chemical properties of fresh rice bran were shown in section 1.1. The analysis of rice bran oil qualities and its bioactive compounds was shown in section 1.2.

1.1 The proximate analysis of rice bran

The yield of rice bran was 93.3–95.7% after separating the foreign matter such as chaff, debris, gravel and sand. The filth was in range of 4.9–7.0% (Table 11). The proximate analysis of rice bran (2 types) reported that moisture content less than 14%, the protein value was 12.0–15.6%, carbohydrates was 31.1–52.3%, the fat content was 15.0–19.7, ash 6.6–9.9% and crude fiber was 7.0–11.4%, which was contributed to the observation of Orthoefer and Eastmena (2004).

1.2 Oil quality and the quantity of bioactive compounds

The qualities of rice bran oil obtained from different extraction method showed as Table 12. The refractive index, specific gravity, saponification, iodine test, peroxide values were complied with Thai Industrial Standard for rice bran oil consumption (TISI, 44–2516). For water and volatile matter, the color by Lovibond, unsaponification matter, acid value of oil extracted by solvent was higher than cold pressed extraction (Table 12–13). For the tocopherols, oryzanol, total phytosterols of oil extracted by cold pressed was higher than solvent extraction. Moreover, the results of fatty acid analysis by using chromatography technique showed by the ratio of SFA: MUFA: PUFA as 23.4: 45.2: 31.3 (cold pressed extraction) and 23.4: 45.2: 31.3 (solvent extraction) (Table 14–15).

Table 11 The percentage of yield and proximate analysis of rice bran.

Characterization	Khao–dawk–mali 105	Mixed rice bran
1. Yield (%)		
- Rice bran	93.30 ± 1.4	95.70 ± 2.1
- Filth or residual	6.70 ± 1.4	4.30 ± 2.1
- Total	100.0	100.0
2. Proximate analysis (g/100g.)		
- Moisture	9.93 ± 0.2	10.84 ± 0.1
- Protein	12.15 ± 0.1	13.50 ± 0.1
- Ash	11.70 ± 0.3	8.56 ± 0.2
- Crude fiber	9.79 ± 0.1	6.84 ± 0.2
- Fat	24.16 ± 0.4	18.65 ± 0.3
- Carbohydrate	42.06 ± 0.6	48.45 ± 0.5

According to the result from Table 12, the volatile matter content in the oil sample extracted by solvent extraction had higher content than standard limit (TISI, 2516) as there is still some hexane left in the oil sample. This is because the limitation of vacuum evaporator used here cannot control the evaporator of hexane under the pressure at 200 mbar. Furthermore, the standard of color specified that the Y +5R value should not more than 20 while the color of oil sample showed the value over 40. The high value of color related with the unremoved of pigment and the chlorophyll compound in crude rice bran oil.

As the standard limitation, unsaponification matter of oil should not more than 3.0, while the analyzed value showed the ranged from 3.25–3.53 which was contributed to the extraction process. The condition of oil extraction and the purify process affected the compound in crude oil (e.g., tocopherol, tocotrienol, phytosterol, gamma oryzanol) such as high temperature and high pressure factor could have eliminate all of those soluble compounds.

Table 12 Qualities of rice bran oil obtained from solvent extraction.

Characterizations	Crude oil Khao–dawk–mali 105	Crude oil Mixed rice bran	Commercial rice bran oil
- Water & volatile matter (%)	1.52±0.0	2.03±0.0	0.1
- Color (Y+5R)	89.00±7.3	51.00±2.5	35.0
- Refractive index	1.46±0.0	1.46±0.0	–
- Specific gravity	0.90±0.0	0.90±0.0	–
- Saponification value (mg KOH/g oil)	188.89±0.4	185.64±0.9	180–195
- Unsaponification matter (%)	3.45 ± 0.3	3.25±0.3	–
- Acid value (mg KOH/g oil)	27.05 ± 3.0	25.88±4.7	0.3
- Iodine value (Wijs)	101.47 ± 0.5	104.29±1.9	92–115
- Peroxide value (milliequivalent/Kg oil)	7.50 ± 1.1	16.35±5.7	1.5

In case of the acid value, all of samples had higher content than the standard guidance (< 0.6). The important factor which effects to the oil quality is enzyme lipase that could accelerate the hydrolysis reaction. The products of that reaction are mono–, diacylglycerol, and free fatty acid (FFA). When FFA increased the qualities of oil decreased. The main factor effects on the acid value in the oil are the inactivated enzyme method, storage temperature, the humidity and the packaging (Orthoefer and Eastman, 2004). Furthermore, The AOCS method used to determine FFA have to be modified since the recommended indicator (phenolphthalein) leads to an overestimation of the FFA content. This is because the phenolic hydroxyl group of the oryzanol causes a shift in the acid–base equilibrium. An indicator with a different pH working range was therefore selected (bromothymol blue).

Table 13 Qualities of rice bran oil obtained from cold pressed extraction.

Characterizations	Crude oil Khao–dawk–mali 105	Crude oil Mixed rice bran
- Water & volatile matter (%)	0.03±0.0	0.06±0.01
- Color (Y+5R)	43.37±0.92	83.83±7.22
- Refractive index	1.46±0.00	1.46±0.00
- Specific gravity	0.90±0.00	0.90±0.00
- Saponification value (mg KOH/g oil)	190.05±1.34	190.10±0.84
- Unsaponification matter (%)	3.53±0.69	3.25±0.84
- Acid value (mg KOH/g oil)	14.35±3.92	21.63±8.98
- Iodine value (Wijs)	97.89±1.03	102.67±0.09
- Peroxide value (milliequivalent/Kg oil)	8.17±2.72	9.37±3.08

An overview of the bioactive compounds in crude rice bran showed in Table 14 and 15. The phytosterol content of Khao–dawk–mali 105 by cold pressed was higher than commercial mixed bran that are complied with Piironen *et al.* (2000). Sitosterol is more higher than campesterol followed by stigmasterol (sitosterol > campesterol > stigmasterol). The quantities of the oryzanol, tocotrienol and tocopherol of mixed–rice bran oil was slightly higher than Khao–dawk–mali 105 rice bran oil.

In addition, the fatty acid composition of all rice bran oil samples had the highest amount of MUFA (23–24%), followed by PUFA (31–33%) and the lowest SFA (42–45%) which was the same content of fat intake (per day) recommendation by the NCEP (the National Cholesterol Education Program) (Naivikul *et al.*, 2008).

Table 14 The content of bioactive compounds in crude oil under solvent extraction.

Characterizations	Crude oil Khao–dawk–mali 105	Crude oil Mixed rice bran
Total phytosterol (ppm.)	15,509.94±464.1	13,571.97±140.5
- β–sitosterol	6,592.5±326.9	3,233.45±83.8
- Stigmasterol	2,932.07±49.8	4,673.64±117.0
- Campesterol	5,985.37±189.2	5,664.88±49.1
% oryzanol	2.04	1.38
Tocopherol (ppm)	87.25	195.02
Tocotrienol (ppm)	408.45	427.15
Fatty acid composition (%)		
-Saturated Fatty Acid	23.00	24.33
14:0 Myristic acid	0.27	0.32
16:0 Palmitic acid	19.62	20.31
17:0 Heptadecanoic acid	0.03	0.04
18:0 Stearic acid	1.72	2.08
20:0 Arachidic acid	0.73	0.84
22:0 Behenic acid	0.23	0.28
24:0 Lignoceric acid	0.40	0.46
-Monounsaturated Fatty Acid	45.42	42.60
16:1 Pamitoleic acid	0.17	0.14
18:1 Oleic acid	44.81	42.01
18:1 Elaidic acid	–	–
20:1 Eicosenoic acid	0.44	0.45
-Polyunsaturated Fatty Acid	32.84	31.56
18:2 Linoleic acid	31.86	30.34
18:3 Linolenic acid	0.93	0.96

Table 15 The content of bioactive compounds in crude oil under cold pressed extraction.

Characterizations	Crude oil Khao–dawk–mali 105	Crude oil Mixed rice bran
Total phytosterol (ppm.)	14,662.12±297.3	12,706.94±60.3
- β–sitosterol	6,837.84±264.3	4069.60±14.9
- Stigmasterol	2,798.27±4.1	5013.65±32.1
- Campesterol	5026.01±68.1	3623.69±22.8
% oryzanol	1.06	1.37
Tocopherol (ppm)	187.03	195.42
Tocotrienol (ppm)	485.29	476.38
Fatty acid composition (%)		
-Saturated Fatty Acid	23.48	24.09
14:0 Myristic acid	0.23	0.32
16:0 Palmitic acid	19.81	20.14
17:0 Heptadecanoic acid	0.04	0.04
18:0 Stearic acid	1.94	2.03
20:0 Arachidic acid	0.8	0.82
22:0 Behenic acid	0.25	0.28
24:0 Lignoceric acid	0.41	0.46
-Monounsaturated Fatty Acid	45.24	42.43
16:1 Pamitoleic acid	0.17	0.15
18:1 Oleic acid	44.61	41.84
18:1 Elaidic acid	–	–
20:1 Eicosenoic acid	0.46	0.44
-Polyunsaturated Fatty Acid	31.28	33.49
18:2 Linoleic acid	30.21	32.33
18:3 Linolenic acid	0.86	0.96

From this study, the qualities of crude rice bran oil from Khao–dawk–mali 105 and mixed–bran by different methods were compared. Crude rice bran oil by cold pressed extraction had high amount of bioactive compounds (e.g., total phytosterol, oryzanol, tocopherol) and also had the standard quality for the consumption as required by Thai Industrial Standards Institute (TISI, 2516). Furthermore, crude oil from Khao–dawk–mali 105 had lighter color than mixed–bran crude oil. Hence, Khao–dawk–mali 105 extracted by cold pressed was selected for study the utilization of crude rice bran oil.

2. The stability of rice bran oil in water emulsion

In this section, commercial rice bran oil was used as the disperse phase for the study of the effect of biopolymer types on the stability of oil in water emulsion to create highly stable system. Moreover, the influence of environmental stresses (pH, ionic strength and temperature) on the stability of the emulsion stabilized by food grade biopolymer was also investigated. It divided into 3 sections which was explored the suitable mount of emulsifier for creating stable emulsion first (section 2.1). Then, the effect of the environmental stresses on emulsion stability (section 2.2) and the oxidation stability of rice bran oil emulsion (section 2.3) were investigated, respectively.

2.1 The influence of biopolymer type on emulsion formation

The purpose of these experiments was to establish the minimum amount of each type of biopolymer emulsifier that could be used to prepare stable emulsions. Emulsions were prepared by homogenizing 5% rice bran oil with 95% aqueous phases containing different emulsifier types and concentrations: WPI (0.02–5.0 wt%); GA (0.05–10.0 wt%); MS (0.2–5.0 wt%). The dependence of the mean droplet diameter (d_{43}) of the resulting emulsions on initial emulsifier concentration was then measured 6 h after homogenization. The mean droplet diameter tended to decrease as the emulsifier concentration was increased (Figure 17), which can be attributed to the fact that there was more emulsifier available to cover the newly formed oil–water interfaces created during homogenization, as well as to the fact that the interfaces become saturated more rapidly at higher emulsifier concentrations (Walstra, 1993, 2003; Jafari *et al.*, 2008). Figure 17 showed that the mean droplet diameter decreased steeply with emulsifier concentration up to a certain emulsifier level (Region I), but then it only changed gradually when the emulsifier concentration was increased further (Region II). In Region I, the droplet size is limited by the amount of emulsifier available to cover the droplets formed, but in Region II the droplet size is mainly limited by the maximum disruptive forces generated by the homogenizer (Tcholakova *et al.*, 2003, 2004). The emulsifier concentration demarking Region I and II was around 0.25% for WPI, 5.0% for GA and 0.5% for MS. The minimum droplet diameter that could be produced also depended on emulsifier type and concentration, *e.g.,* at 4 wt% emulsifier, d_{43} = 0.28, 0.38 and 1.1 μm, MS, WPI and GA, respectively. In the remainder of the experiments rice bran oil emulsions were prepared using emulsifier concentrations that were capable of producing the small droplet sizes without having too much excess emulsifier present, *i.e.,* 0.45% WPI, 1.0% MS and 10.0% GA.

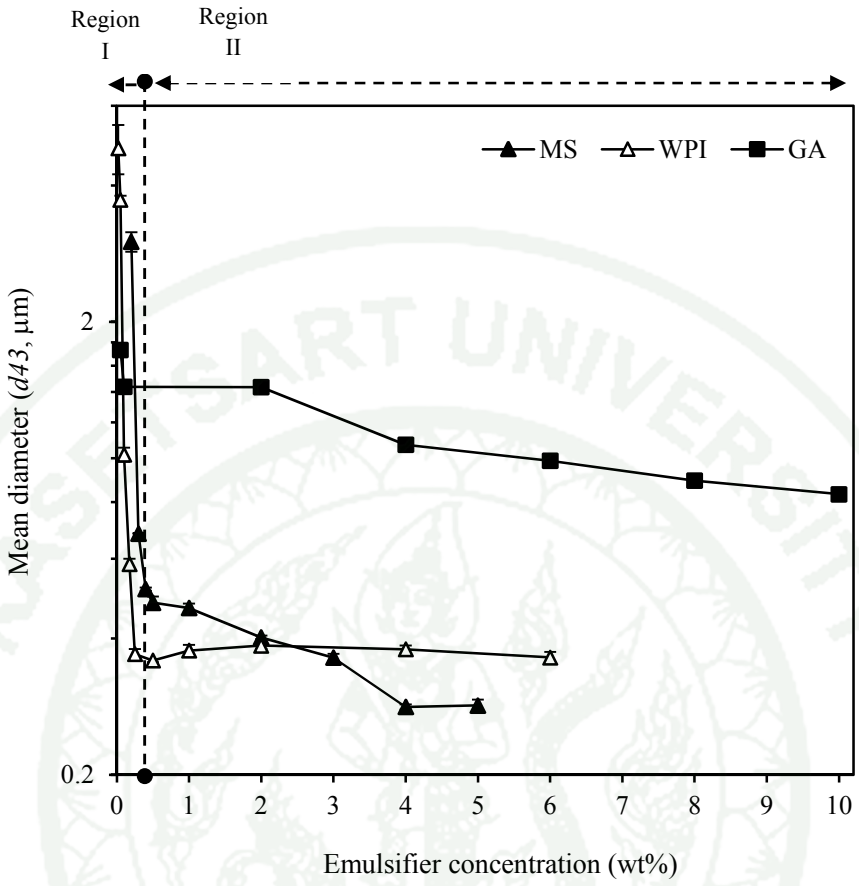


Figure 17 Influence of emulsifier concentration on the mean particle diameter of diluted 5% rice bran oil–in–water emulsions stabilized by whey protein isolate (WPI), gum arabic (GA) or modified starch (MS).

In commercial applications, the optimum amount of a specific emulsifier required would have to be determined for the particular set of product compositions and homogenization conditions utilized. Indeed, recent studies have shown that the molecular characteristics of GA are altered during homogenization by an amount that depends on the homogenization pressure, which may affect its performance as an emulsifier (Al–Assaf *et al*., 2009). Clearly, further study is required to identify the influence of varying homogenization conditions on the molecular characteristics and performances of other biopolymer emulsifiers.

2.2 Influence of environmental stress on rice bran oil emulsion stability

2.2.1 pH effect on emulsion stability

Commercially, rice bran oil may be used in emulsified products with different aqueous phase pH values, e.g., soft drinks tend to be acidic, whereas infant formulas tend to be neutral. Hence, the influence of pH on the physicochemical properties of rice bran oil in water emulsions stabilized by WPI, GA and MS were examined. The mean droplet diameter (d_{43}) of the WPI–stabilized emulsions was around 0.53 μm at relatively low (pH 3) and high (pH 6 to 8) pH values (Figure 18).

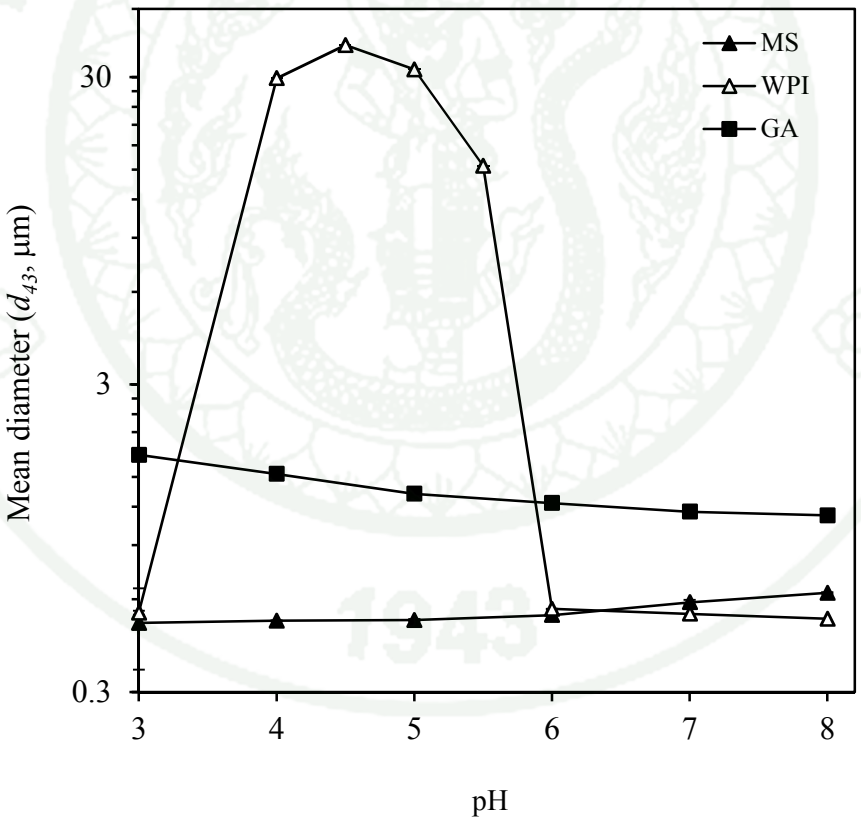
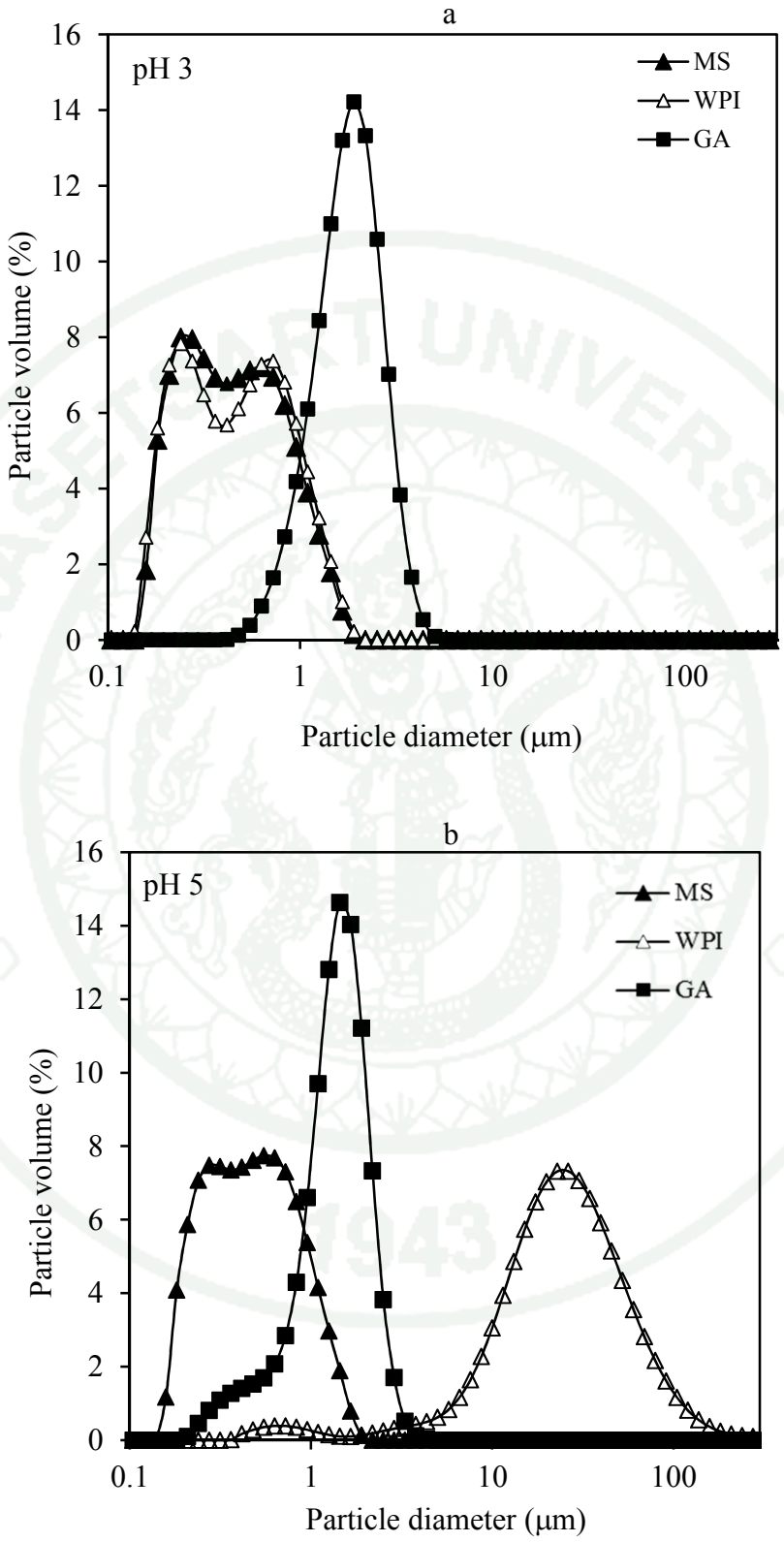


Figure 18 Dependence of the mean droplet diameter (d_{43}) of diluted 5% rice bran oil–in–water emulsions stabilized by whey protein isolate (WPI), gum arabic (GA) and modified starch (MS) on pH.

Nevertheless, a large increase in mean particle diameter was observed around the isoelectric point (4 < pH < 6) of the WPI. On the other hand, the mean droplet diameter of GA–stabilized emulsions (d_{43} ≈ 1.2–1.8 μm) and MS–stabilized emulsions (d_{43} ≈ 0.50–0.69 μm) remained relatively constant from pH 3 to 8 (Figure 18).

Light scattering measurements indicated that there was little pH–dependence of the particle size distribution of the GA– or MS–stabilized emulsions, but that extensive aggregation occurred in the WPI–stabilized emulsions at pH 5 (Figure 19). The particle size measurements were supported by creaming stability measurements, which indicated that WPI–stabilized emulsions were highly unstable to creaming at pH values around their pI but stable at higher and lower pH values, whereas GA– and MS–stabilized emulsions were stable across the entire pH range studied (Figure 20). These results are in agreement with earlier studies of the pH–stability of biopolymer–stabilized oil–in–water emulsions (Demetriades *et al.*, 1997b; Kulmyrzaev *et al.*, 2000; Chanamai and McClements, 2002). The poor stability of the WPI–stabilized emulsions around the protein's isoelectric point (pI) can be attributed to a reduction in the electrostatic repulsion between the oil droplets, which leads to droplet flocculation (McClements, 2005). The good pH–stability of the MS– and GA–stabilized emulsions can be accounted for by the fact that the lipid droplets are coated by a relatively thick layer of hydrophilic polysaccharide molecules, and hence they are largely stabilized by steric repulsion rather than electrostatic repulsion (Chanamai and McClements, 2002; McClements, 2005). In addition, the presence of the thick polysaccharide layer also decreases the magnitude of the attractive van der Waals forces acting between the droplets, which increases emulsion stability to flocculation (Guzey and McClements, 2007).



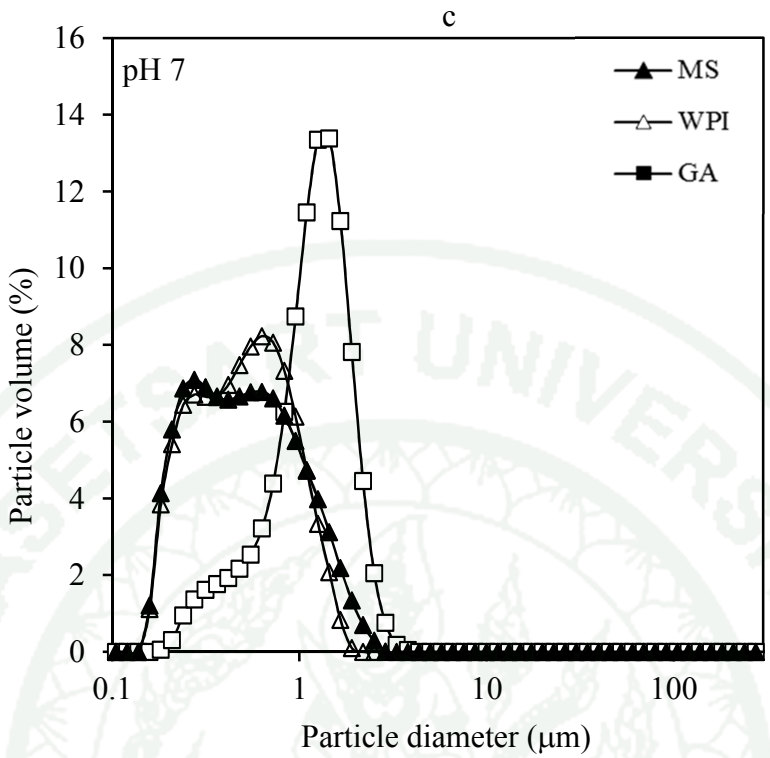


Figure 19 Particle size distributions of diluted 5% rice bran oil–in–water emulsions stabilized by whey protein isolate 0.45% (WPI), gum arabic 10.0% (GA) or modified starch 1.0%(MS) at (a) pH 3; (b) pH 5; (c) pH 7.

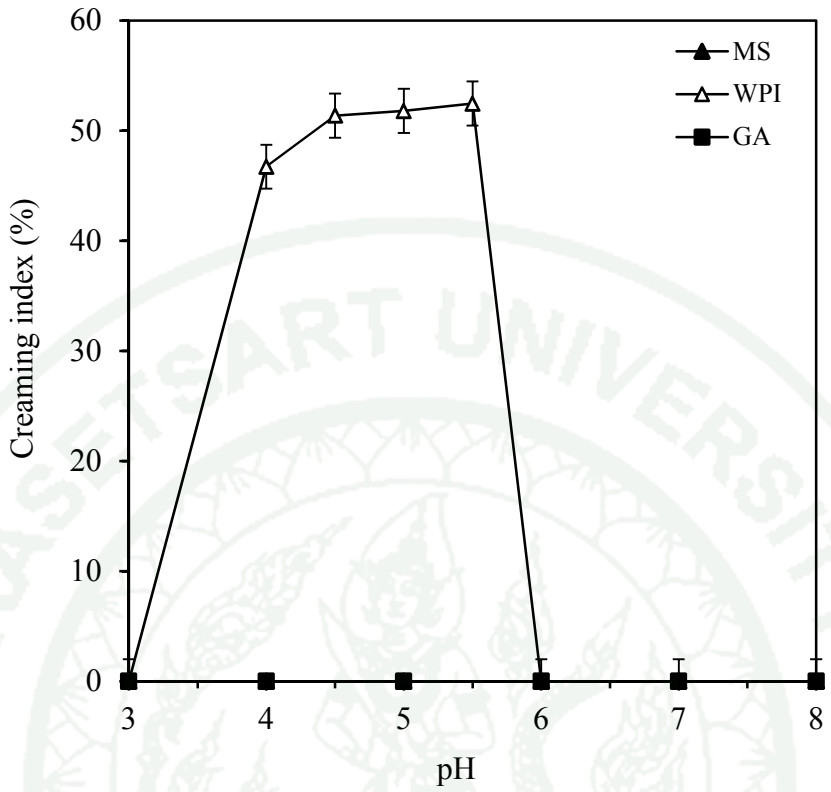


Figure 20 pH–dependence of the creaming index of 5% rice bran oil–in–water emulsions stabilized with whey protein isolate (WPI), gum arabic (GA) and modified starch (MS).

The pH–dependence of the droplet ζ–potential for the three types of emulsion is shown in Figure 21. The ζ–potential of the WPI–stabilized droplets went from highly positive at low pH to highly negative at high pH, with a point of zero charge around pH 4.6. This pH dependence of the droplet charge is due to the fact that the isoelectric point of adsorbed layer of WPI molecules is around pH 5 (Demetriades *et al.*, 1997b; Demetriades and McClements, 1998; Kulmyrzaev *et al.*, 2000; Chanamai and McClements, 2002). At relatively high H^+ concentrations (pH << pI), the amino groups are positively charged ($–NH_3^+$) and the carboxyl groups are neutral (–COOH) so the net protein charge is positive. At relatively low H^+ concentrations (*i.e.,* pH >> pI), the carboxyl groups are negatively charged ($–COO^-$) and the amino groups are neutral ($–NH_2$) so the net protein charge is negative. At the pI, the number

of positively and negatively charged groups on the protein is balanced and so the protein has no net charge. The interfacial layers formed by globular proteins tend to be relatively thin (a few nanometers thick) and so this type of biopolymer emulsifier tends to stabilize emulsions mainly by electrostatic repulsion rather than steric repulsion. Hence, when the protein loses its net charge around the pI the stability of the droplets to aggregation is greatly reduced since the attractive van der Waals forces then dominate.

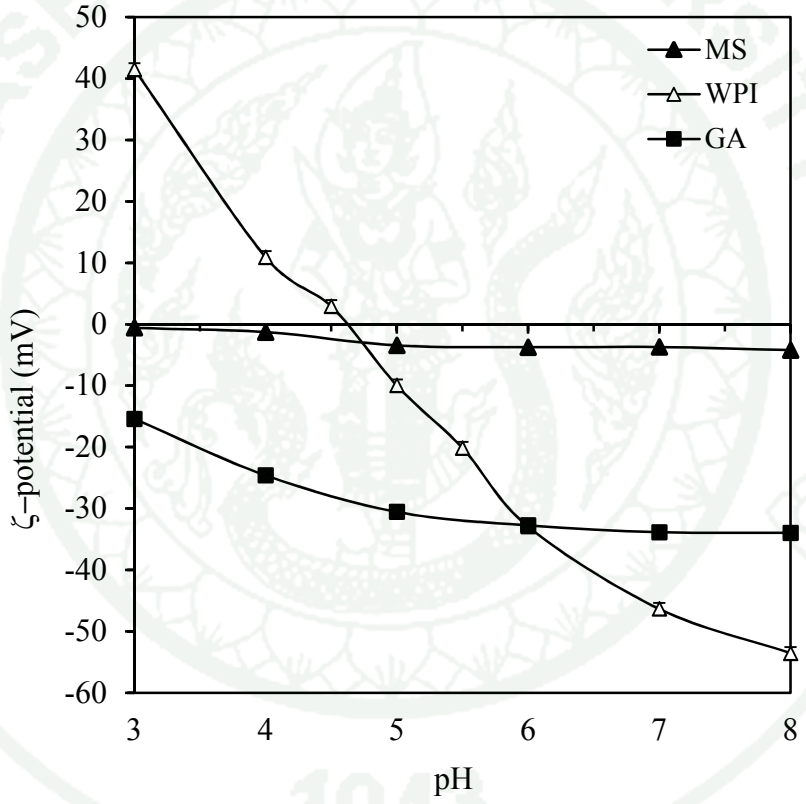


Figure 21 pH–dependence of particle electrical charge (ζ–potential) of diluted 5% rice bran oil–in–water emulsions stabilized with whey protein isolate (WPI), gum arabic (GA) and modified starch (MS).

The ζ–potentials of the lipid droplets stabilized by GA and MS were negative at all pH values (Figure 21), which can be attributed to the presence of some negatively charged side groups ($–COO^-$) on these polysaccharide molecules (Ray *et al.*, 1995; Tan, 1997; Padala *et al.*, 2009). Interestingly, the GA had a much higher negative charge than the MS at all pH values, which suggests that the linear charge density of the gum arabic was higher than the modified starch. This may have important consequences for the interactions of biopolymer–stabilized lipid droplets with other charged species in food and beverage systems, such as transition metals that promote lipid oxidation. For example, it has been shown that negatively charged droplets attract positively charged transition metals to lipid droplet surfaces, which promotes lipid oxidation (McClements and Decker, 2000; Hu *et al.*, 2003b). There was a slight reduction in the negative charge on the GA– and MS–stabilized lipid droplets when the pH was reduced below about 5, which can be attributed to the fact that this solution pH moved around and below the pK_a values of the carboxyl groups so that they lost some of their negative charge.

2.2.2 Ionic strength on emulsion stability

The ionic strength of emulsified foods and beverages may vary considerably depending on the nature of the food products that the lipid droplets are incorporated into. The influence of ionic strength (0 to 500 mM NaCl) on the stability of rice bran oil emulsions stabilized by the three different kinds of biopolymer emulsifier was examined.

In the absence of salt, the mean droplet diameters (d_{43}) of the emulsions were initially ≈ 0.68, 1.05 and 0.54 μm for WPI, GA and MS, respectively. There was little change in the particle size of the emulsions stabilized by gum arabic or modified starch with increasing ionic strength (Figure 21), which can be attributed to the fact that these emulsions are primarily stabilized by steric repulsion, rather than electrostatic interactions (McClements, 2005). On the other hand, there was an appreciable increase in the mean particle diameter of the WPI–stabilized emulsions at ionic strengths of 200 mM and higher (Figure 22). This increase in droplet

aggregation at higher salt concentrations is due to screening of the electrostatic repulsion between the protein–coated droplets (McClements, 2005). Above a critical salt level, the electrostatic repulsion is no longer strong enough to overcome the attractive interactions (van der Waals and hydrophobic) acting between the droplets. Visual observations of the emulsions containing different salt levels indicated that a distinct cream layer formed on top of the WPI–stabilized emulsions at higher salt levels (≥ 200 mM), but that the rest of the emulsions were relatively stable to gravitational separation.

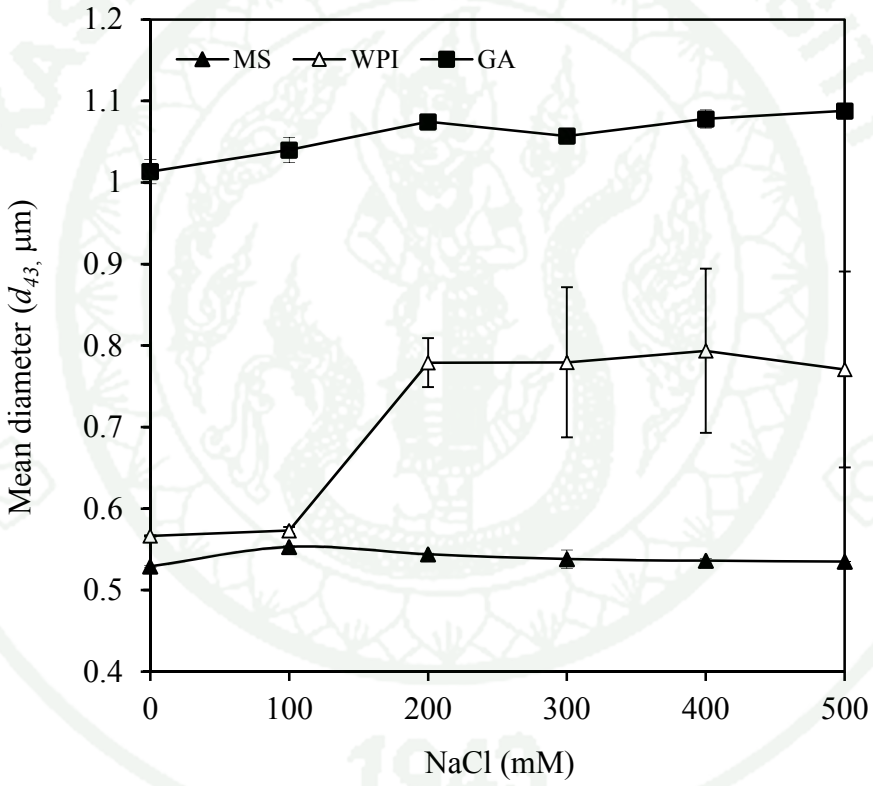


Figure 22 Ionic strength dependence of the mean particle diameter (d_{43}) of diluted 5% rice bran oil–in–water emulsions stabilized with whey protein isolate (WPI), gum arabic (GA) and modified starch (MS).

For all three emulsifiers, there was a decrease in the magnitude of the negative ζ–potential with increasing salt concentration (Figure 23), which can be attributed to electrostatic screening effects (Israelachvili, 1992). Counter–ions (Na^+) in the aqueous phase accumulate around the negatively charged groups ($–COO^-$) on the protein surface due to electrostatic attraction, thereby reducing their net charge (McClements, 2005).

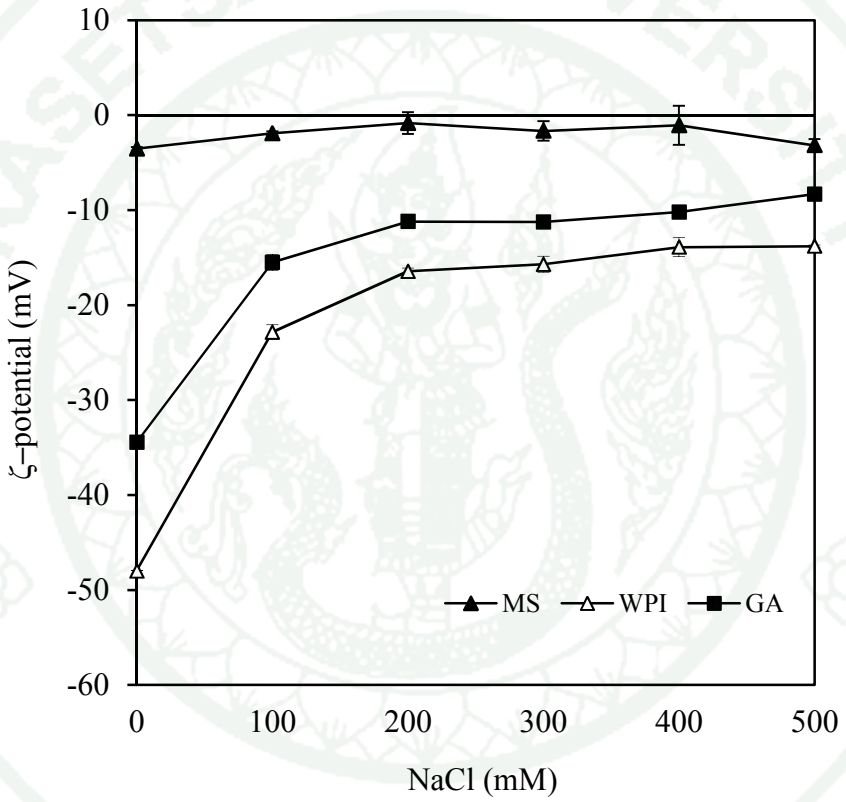


Figure 23 Dependence of particle electrical charge (ζ–potential) of diluted 5% rice bran oil in water emulsions stabilized with whey protein isolate (WPI), gum arabic (GA) and modified starch (MS) on salt concentration (pH 7).

2.2.3 Thermal processing on emulsion stabilty

Many emulsified food products go through some type of thermal process during their manufacture or utilization, e.g., sterilization, pasteurization or cooking. The influence of heat treatment (30–90 °C, 30 min) and salt concentration

(0 or 150 mM NaCl) on the particle size (d_{43}), ζ–potential and creaming stability of rice bran oil emulsions stabilized by WPI, GA and MS at pH 7 was investigated. The NaCl was added to the emulsions before they were subjected to heat treatment, since this has previously been shown to have the biggest negative impact on emulsion stability for globular protein stabilized emulsions (Kim *et al.*, 2002, 2005). The particle size was measured after the emulsions had been stored at room temperature overnight, whereas the creaming stability was measured after they had been stored at room temperature for 7 days.

In the absence of added salt, all the emulsions were relatively stable to droplet aggregation and creaming after heat treatments with little change in mean particle diameter and no visible evidence of phase separation (data not shown). For example, after heat treatments ranging from 30 to 90 ºC, the mean droplet diameters (d_{43}) of the emulsions were ≈ 0.58 ± 0.03, 1.07 ± 0.01 and 0.58 ± 0.08 μm for WPI, GA and MS, respectively. The only sample that showed an appreciable increase in mean particle diameter in the absence of salt was for the MS–stabilized emulsion heated at 90 ºC for 30 minutes: d_{43} increased from 0.53 μm before heating to 0.78 μm after heating.

In the presence of added salt (150 mM), the WPI stabilized emulsions became unstable to droplet aggregation when they were heated above 60 °C, as demonstrated by an appreciable increase in mean particle diameter (Figure 24) and some visible evidence of creaming (data not shown). The instability of the WPI–emulsions to heating in the presence of salt can be attributed to thermal denaturation of the globular proteins adsorbed to the lipid droplet surfaces (Kim *et al.*, 2002, 2004, 2005). When the WPI molecules unfold they expose non–polar groups to the surrounding aqueous phase, which increases the surface hydrophobicity of the droplets and promotes aggregation through hydrophobic attraction (Kim *et al.*, 2005). In addition, sulfhydryl groups are also exposed when the protein is heated above its thermal denaturation temperature, which promotes droplet–droplet aggregation through covalent disulfide bonds (Monahan *et al.*, 1996). In the absence of salt, the

electrostatic repulsion is strong enough to overcome the hydrophobic and van der Waals attraction, but in the presence of salt the additional hydrophobic attraction associated with protein unfolding promotes droplet aggregation (McClements, 2005).

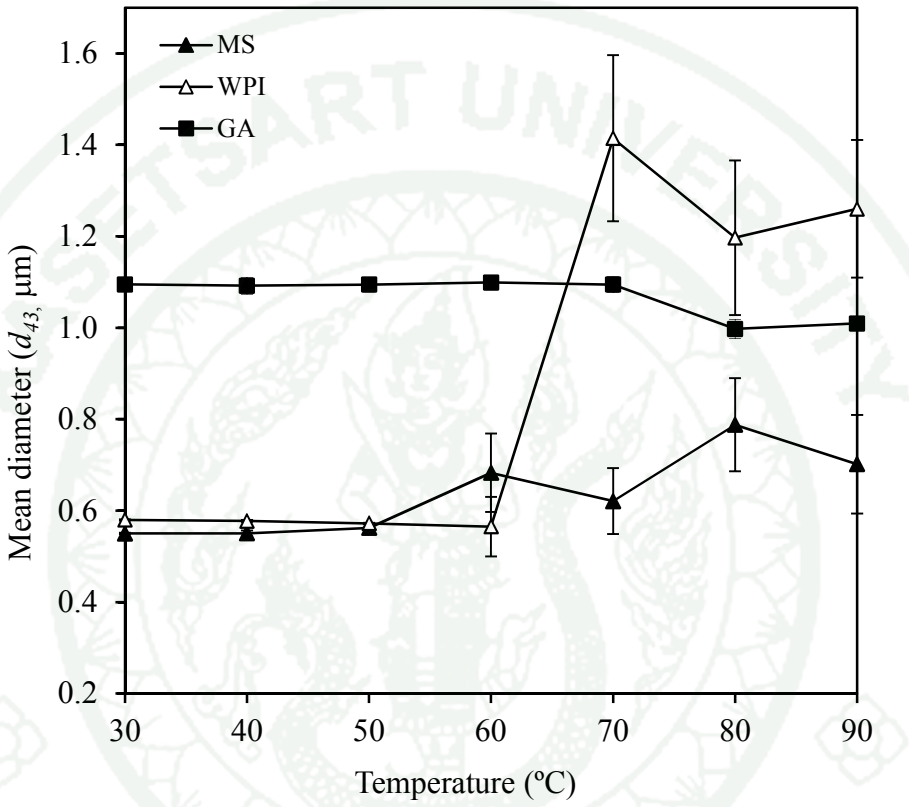


Figure 24 Dependence of the mean droplet diameter (d_{43}) of diluted 5% rice bran oil–in–water emulsions stabilized with whey protein isolate (WPI), gum arabic (GA) and modified starch (MS) on heat treatment (30–90 °C, 30 min, 150 mM NaCl, pH 7).

The GA–and MS–stabilized rice bran oil emulsions did not exhibit extensive droplet aggregation at either 0 or 150 mM NaCl (Figure 24), which can be attributed to the fact that they are stabilized primarily by polysaccharides that do not unfold to expose non–polar groups at higher temperatures. There was a slight increase in the mean particle diameter of the MS–stabilized emulsions above about 60 ºC (Figure 24), but no evidence of creaming (data not shown). This effect may be

attributed to other changes in the molecular characteristics of starch upon heating. Martinez *et al*. (2003) examined the influence of heating on the particle size distributions of emulsions stabilized by modified starch. They found that heating starch–stabilized emulsions around 72–83 °C, which was reported to be the swelling region of starch granules, led to an increase in particle aggregation (Martinez *et al.*, 2003). The electrical characteristics of all of the emulsions were unchanged by heating (data not shown). For example, the ζ–potentials were –48.8 ± 0.6, –31.2 ± 0.6 and –3.5 ± 0.2 mV for WPI, GA and MS, respectively after thermal treatments ranging from 30 to 90 ºC.

2.3 The effect of biopolymer type at different pH (3,7) on oxidation stability of rice bran oil emulsion

Rice bran oil has a relative high concentration of unsaturated fatty acid so it is susceptible to lipid oxidation. The oxidative deterioration of lipid affects the quality of foods by influencing flavor, odor and nutritive value. Furthermore, the selection of food grade biopolymer, such as proteins and polysaccharides, can be used to create a diverse range of delivery systems suitable for encapsulating and protecting the deterioration of lipid. Therefore, the hypothesis of this section is that the oxidative stability of emulsified rice bran oil would depend on the nature of the biopolymer emulsifier coating the lipid droplets due to differences in the composition, structure and properties of the interfacial layer. The aim of this study was therefore to test this hypothesis by comparing the oxidative stability of lipid droplets stabilized by different biopolymer emulsifiers at pH 3 and 7.

In this study, the influence of three different surface–active biopolymers on the oxidative stability of emulsified rice bran oil was examined. These biopolymers were selected based on differences in their molecular characteristics: whey protein isolate is a mixture of amphoteric globular proteins (Dalgleish, 1997; Wilde, 2000); gum arabic is a mixture of anionic polysaccharides and protein fractions (Garti and Leser, 2001; Dickinson, 2003; Al–Assaf and Phillips, 2008) modified starch consists of starch molecules that have been chemically reacted with

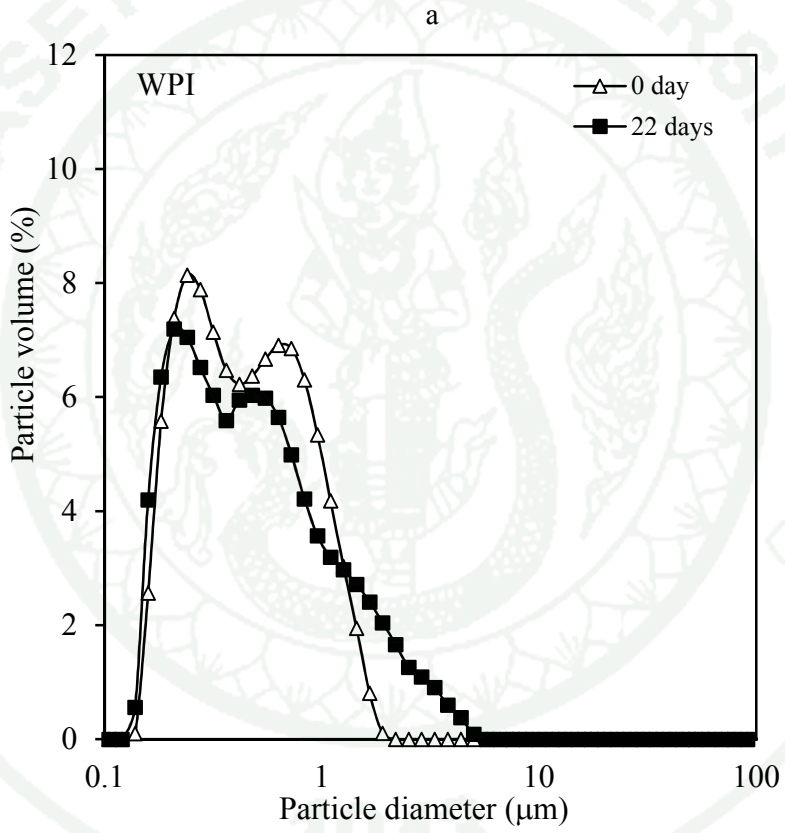
octenyl succinic anhydride (OSA) to give them some hydrophobic characters (Trubiano, 1995; Tan, 2004; Given, 2009). The previous study (3.1–3.2) showed that these three biopolymers could be used to successfully form and stabilize rice bran oil–in–water emulsions.

2.3.1 Emulsion formation and physicochemical properties

The purpose of these experiments was to examine the influence of biopolymer type on the physical properties of rice bran oil–in–water emulsions. The emulsions were prepared using similar conditions as used in previous study (part 2.1 and 2.2): 5 wt% rice bran oil phase; 95% aqueous phase (0.45% WPI, 1.0% MS or 10.0% GA in 10.0 mM citrate buffer at pH 3 or 7). Pro–oxidant (200 μM ferrous iron–EDTA) was added to the emulsions so as to use similar conditions for physical and chemical stability measurements. Emulsions were then incubated at 37 °C and the mean particle diameter and ζ–potential were measured.

All three biopolymer emulsifiers were capable of forming emulsions containing relatively small droplets, with the majority of droplets being < 1 μm in diameter immediately after homogenization (Figure 25). The initial mean particle diameters (d_{43}) depended on pH and biopolymer type: 0.43 and 0.52 μm at pH 3 and 7 for GA; 0.57 and 0.50 μm at pH 3 and 7 for MS; and, 0.58 and 0.61 μm at pH 3 and 7 for WPI. The mean particle diameter (d_{43}) did not change appreciably during storage, with values averaged over the whole incubation period of: 0.70 ± 0.04 and 0.47 ± 0.05 μm at pH 3 and 7 for GA; 0.61 ± 0.05 and 1.03 ± 0.07 μm at pH 3 and 7 for MS; and, 0.49 ± 0.04 and 0.68 ± 0.07 μm at pH 3 and 7 for WPI. Nevertheless, there was evidence of some growth in particle size after prolonged storage, with the fraction of droplets > 1 μm increasing (Figure 25). In particular, there was an appreciable increase in the fraction of large droplets present in the emulsions stabilized by modified starch (Figure 25b), suggesting that either droplet flocculation or coalescence had occurred. Overall, all of the emulsions were relatively stable to creaming during incubation under the storage conditions used for the oxidation

experiments, with no visible evidence of phase separation after 20 days storage. The stability of the MS– and GA–stabilized droplets can be mainly attributed to the strong steric repulsion between droplets resulting from the relatively thick polymer layer adsorbed to their surfaces (Charoen *et al.*, 2011). On the other hand, the good stability of WPI–stabilized droplets at pH 3 and 7 can be attributed to the relatively strong electrostatic repulsion between the droplets due to their high electrical charge at pH values away from the proteins isoelectric point, pI ≈ 5 (Charoen *et al.*, 2011).



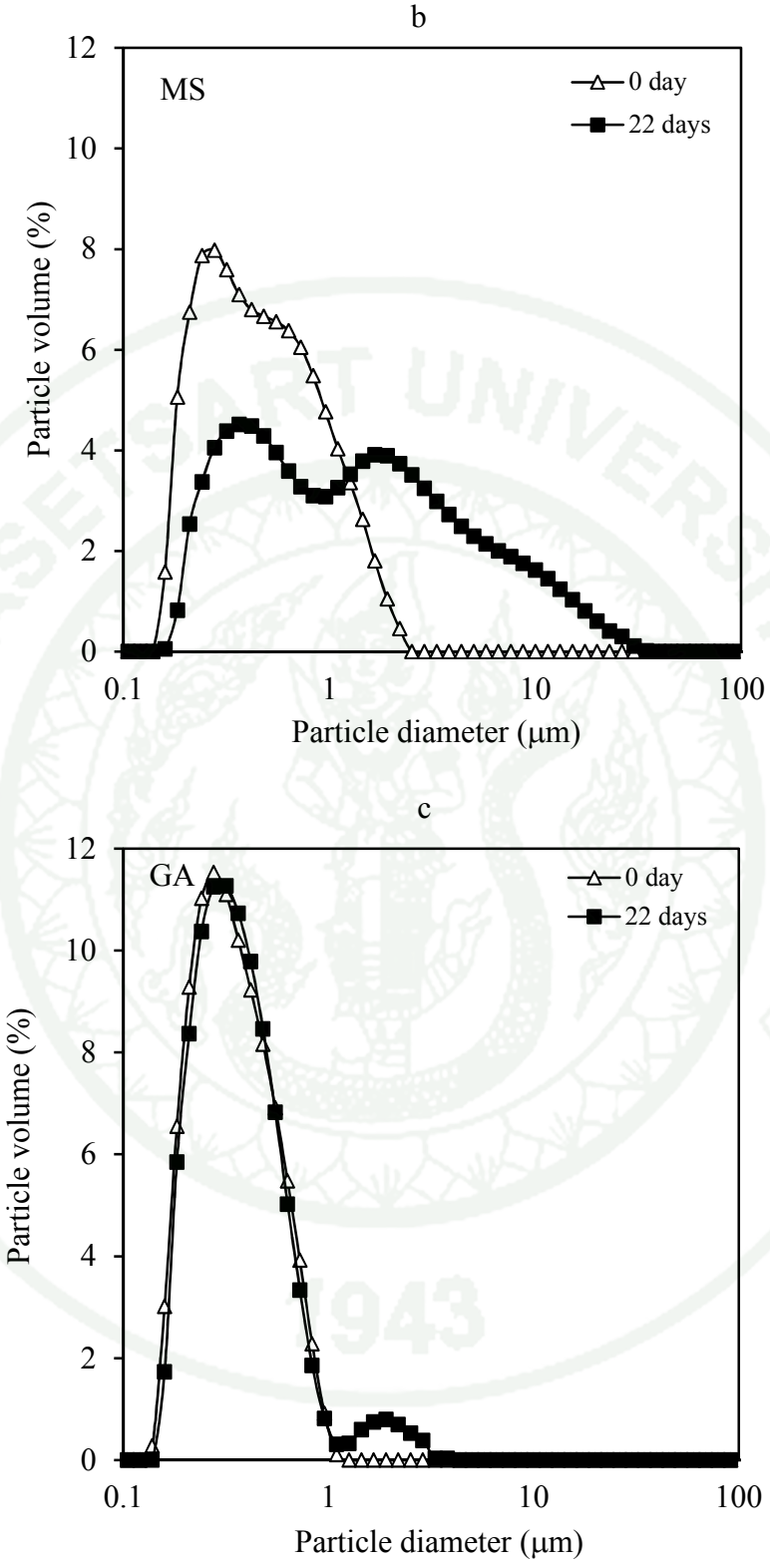


Figure 25 Representative particle size distributions of 5% rice bran oil–in–water emulsions stabilized by (a) 0.45% whey protein isolate; (b) 1.0% modified starch and (c) 10.0% gum arabic at 0 day and 22 days.

The electrical characteristics of emulsions are important because they determine the droplet's stability to aggregation, as well as their interactions with other charged species (such as pro–oxidant cationic transition metal ions). The electrical characteristics on the droplets depended on pH and biopolymer type: –13 and –27 mV at pH 3 and 7 for GA; –2 and –3 mV at pH 3 and 7 for MS; +37 and –38 mV at pH 3 and 7 for WPI (Figure 26). The ζ–potentials of the lipid droplets stabilized by GA and MS were negative at all pH values, which can be attributed to the presence of negatively charged side groups ($–COO^-$) on these polysaccharide molecules (Ray *et al.*, 1995; Tan, 1997; Padala *et al.*, 2009). GA had a much higher negative charge than MS at pH 3 and 7, which suggests that the linear charge density of gum arabic was higher than modified starch. The ζ–potential of the WPI–stabilized droplets went from highly positive at pH 3 to highly negative at pH 7. At relatively high H^+ concentrations (pH << pI), the amino groups are positively charged ($–NH_3^+$) and the carboxyl groups are neutral (–COOH) so the net protein charge is positive. At relatively low H^+ concentrations (*i.e.,* pH >> pI), the carboxyl groups are negatively charged ($–COO^-$) and the amino groups are neutral ($–NH_2$) so the net protein charge is negative (Demetriades *et al.*, 1997b; Kulmyrzaev *et al.*, 2000; Chanamai and McClements, 2002). For all systems, there was little change in the charge on the droplets during storage, *i.e.,* the ζ–potential changed by less than 3 mV for all samples.

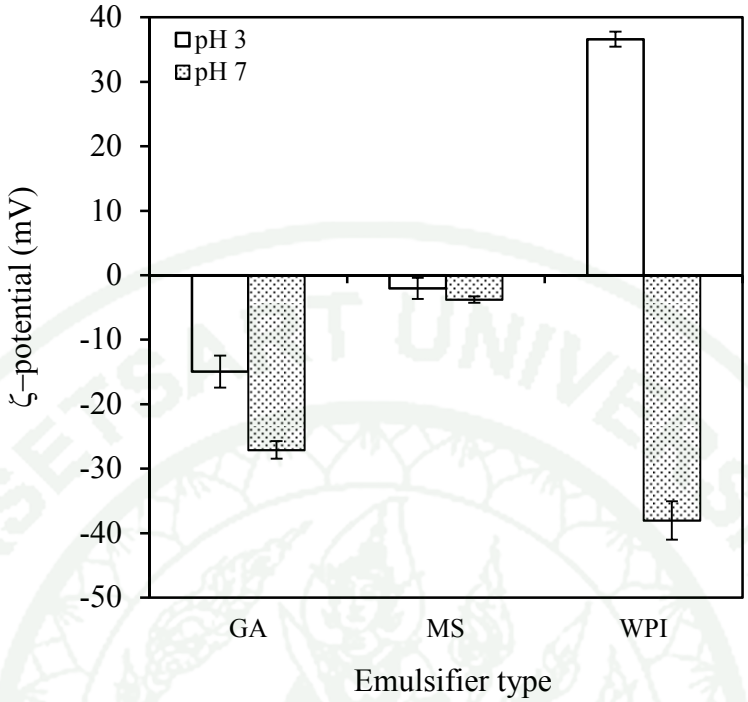


Figure 26 Particle electrical charge (ζ–potential) of 5% rice bran oil–in–water emulsions stabilized by 0.45% whey protein isolate (WPI); 1.0% modified starch (MS) or 10.0% gum arabic (GA) at pH 3 and 7.

2.3.2 Influence of biopolymer type on oxidative stability at pH 3

In this section, the influence of biopolymer type on the oxidative stability of rice bran oil–in–water emulsions was examined under acidic conditions (pH 3) to mimic the acidic environment found in many commercial food and beverage products, e.g., soft drinks, fruit drinks, and salad dressings. Rice bran oil emulsions were prepared at pH 3 using three different surface–active biopolymers: WPI (0.45%); MS (1.0%); and GA (10.0%). The formation of lipid oxidation primary products (peroxides) and secondary products (hexanal) was then monitored during storage at 37 °C in the presence of a pro–oxidant (iron/EDTA complex). Iron is a transition metal that is known to be a highly potent pro–oxidant, but that has relatively poor water solubility. On the other hand, EDTA is a chelating agent that is a highly potent anti–oxidant when used in excess. When used in a 1:1 molar ratio the iron–EDTA

complex is a highly effective pro–oxidant because EDTA increases the water–solubility of the iron but does not bind it in a manner that prohibits its ability to participate in the lipid oxidation reaction.

In all emulsion samples there was an increase in the concentration of hydroperoxides (primary reaction products) and hexanal (secondary reaction products) detected during storage (Figure 27). However, the rate of oxidation clearly depended on biopolymer type, decreasing in the following order: GA > MS >WPI. The relative impact of biopolymer type and pro–oxidant addition on the lipid oxidation rate was compared directly by plotting the total amount of hexanal formed after 12 days of storage (Figure 28). This figure clearly shows that the stability of the emulsified lipids to oxidation was worse in the GA–stabilized emulsions than in the other two emulsions. Previous studies have suggested that the electrical charge on lipid droplets plays a key role in determining their stability to lipid oxidation (McClements and Decker, 2000). It has been proposed that negatively charged lipid droplets attract positively charged transition metals to their surfaces, thereby bringing the pro–oxidant into close proximity to the lipid substrate and increasing the oxidation rate (Hu *et al.*, 2003a, 2003b). This mechanism would account for the relative rate of lipid oxidation observed in the emulsions, since GA–stabilized droplets had a high negative charge, MS–stabilized droplets had a slight negative charge, and WPI–stabilized droplets had a high positive charge (Figure 26).

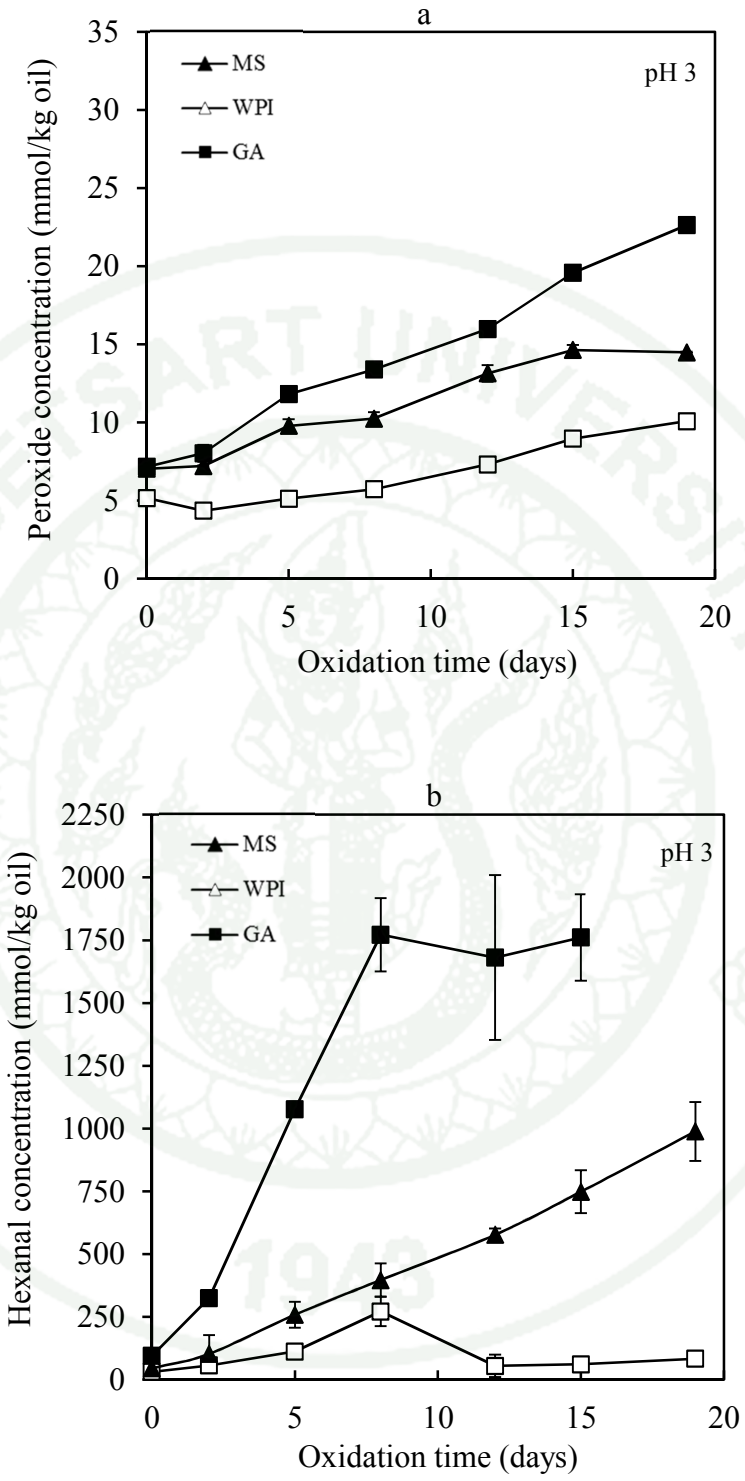


Figure 27 Formation of lipid hydroperoxide concentration (a) and hexanal concentration (b) in 5% rice bran oil–in–water emulsions stabilized by 0.45% whey protein isolate (WPI); 1.0% modified starch (MS) and 10.0% gum arabic (GA) at pH 3 during storage at 37 °C.

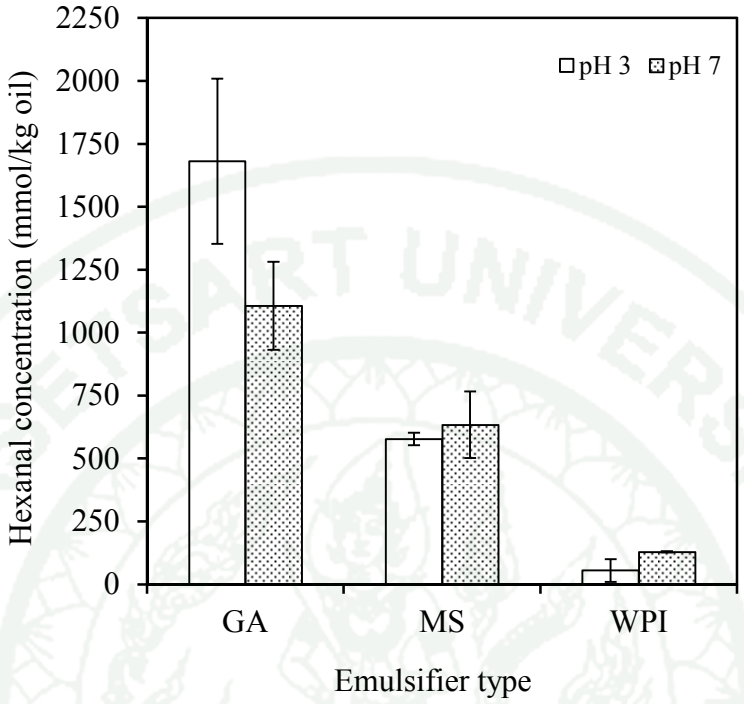


Figure 28 Formation of hexanal concentration in 5% rice bran oil–in–water emulsions stabilized by 0.45% whey protein isolate (WPI); 1.0% modified starch (MS) and 10.0% gum arabic (GA) at pH 3 and pH 7 during storage at 37 °C for 13 days.

Other physicochemical phenomena may also have contributed to the ability of the different biopolymers to alter the oxidation rate. First, the interfacial coatings formed by different biopolymers may have had different abilities to prevent the transition metals from reacting with the emulsified lipids, e.g., due to their thickness, packing, or chemical composition. A thick densely packed coating would be expected to reduce ion diffusion more effectively than a thin openly packed coating. If the iron–binding anionic groups on a biopolymer molecule were located relatively far from the oil–water interface (e.g., by protruding into the aqueous phase), then they may be able to keep the iron ions away from the encapsulated lipid. On the other hand, if the iron–binding anionic groups are in close proximity to the oil–water interface, then they may be able to bring the iron into close proximity to the lipid

thereby promoting oxidation. Second, non–adsorbed anionic polysaccharides may inhibit lipid oxidation in oil–in–water emulsions due to their ability to chelate transition metal ions at negatively charged sites, thereby preventing them from coming into close contact with the lipid phase, e.g., xanthan (Shimada *et al.*, 1992), gum arabic (Matsumura *et al.*, 2003), pectin, carrageenan and alginate (Chen *et al.*, 2010). On the other hand, if the anionic polysaccharide is adsorbed to the droplet surfaces it may promote oxidation by bringing the transition metals close to the lipid phase. Third, some polysaccharides have been claimed to have antioxidant activity due to their ability to donate hydrogen and therefore act as radical chain breakers, e.g., tragacanth (McClements and Decker, 2000). These polysaccharides are likely to be most effective as antioxidants if they are located at the site of the oxidation reaction, *i.e.,* the oil–water interface. Fourth, some anionic polysaccharides have been reported to promote lipid oxidation in emulsions because they contain appreciable amounts of transition metal impurities that act as pro–oxidants (Katsuda *et al.*, 2008). Fifth, globular proteins have been reported to inhibit lipid oxidation in emulsions due to free radical scavenging by sulfhydryl and non–sulfhydryl amino acids, plus some limited transition metal chelation (Tong *et al.*, 2000a, 2000b). Finally, it is possible that the binding of iron ions to anionic polysaccharides may compete with the binding of iron ions to EDTA, which could alter the chemical reactivity of the iron ions.

2.3.3 Influence of biopolymer type on oxidative stability at pH 7

In this section, the influence of biopolymer type on the oxidative stability of rice bran oil emulsions was determined under neutral conditions (pH 7), because many food and beverage products have neutral aqueous phases, such as infant formula, sports drinks, soups, and sauces. Rice bran oil emulsions were prepared at pH 7 using the three different surface active biopolymers: WPI (0.45%); MS (1.0%); and, GA (10.0%). The formation of lipid oxidation products (peroxides and hexanal) was then monitored during storage in the presence of a pro–oxidant (iron/EDTA complex).

There was an appreciable increase in the production of primary and secondary lipid oxidation products in all of the emulsions during storage (Figure 29). The lipid oxidation rate again depended on biopolymer type, following a similar order as observed at pH 3: GA > MS > WPI. The relative impact of biopolymer type on oxidation rate was directly compared by plotting the total amount of hexanal formed after about 12 days of storage (Figure 28), which shows that the oxidation rate is slower in the emulsion containing protein– stabilized droplets than the ones contained polysaccharide–stabilized droplets.

Comparing the lipid oxidation data at different pH values, one can see that the amount of primary reaction products present is higher at pH 7 than pH 3 (Figures 27 and 29a), but the concentration of secondary reaction products is higher at pH 3 than pH 7 (Figures 27 and 29b). A possible explanation for this observation is that the primary products broke down more rapidly at pH 3, thereby lowering the amount remaining. The concentration of primary products measured at a specific time is a balance between primary product formation and degradation. Hence, primary products tend to increase initially, reach a maximum value, and then decrease at longer times. Overall, this results therefore suggest that the oxidation rate is faster at pH 3 than at pH 7. This effect may be attributed to differences in the solubility of endogenous transition metals at different pH values (McClements and Decker, 2000). The solubility of transition metals increases appreciably when the pH is reduced, which may account for the faster rate of secondary reaction production formation at pH 3 than pH 7.

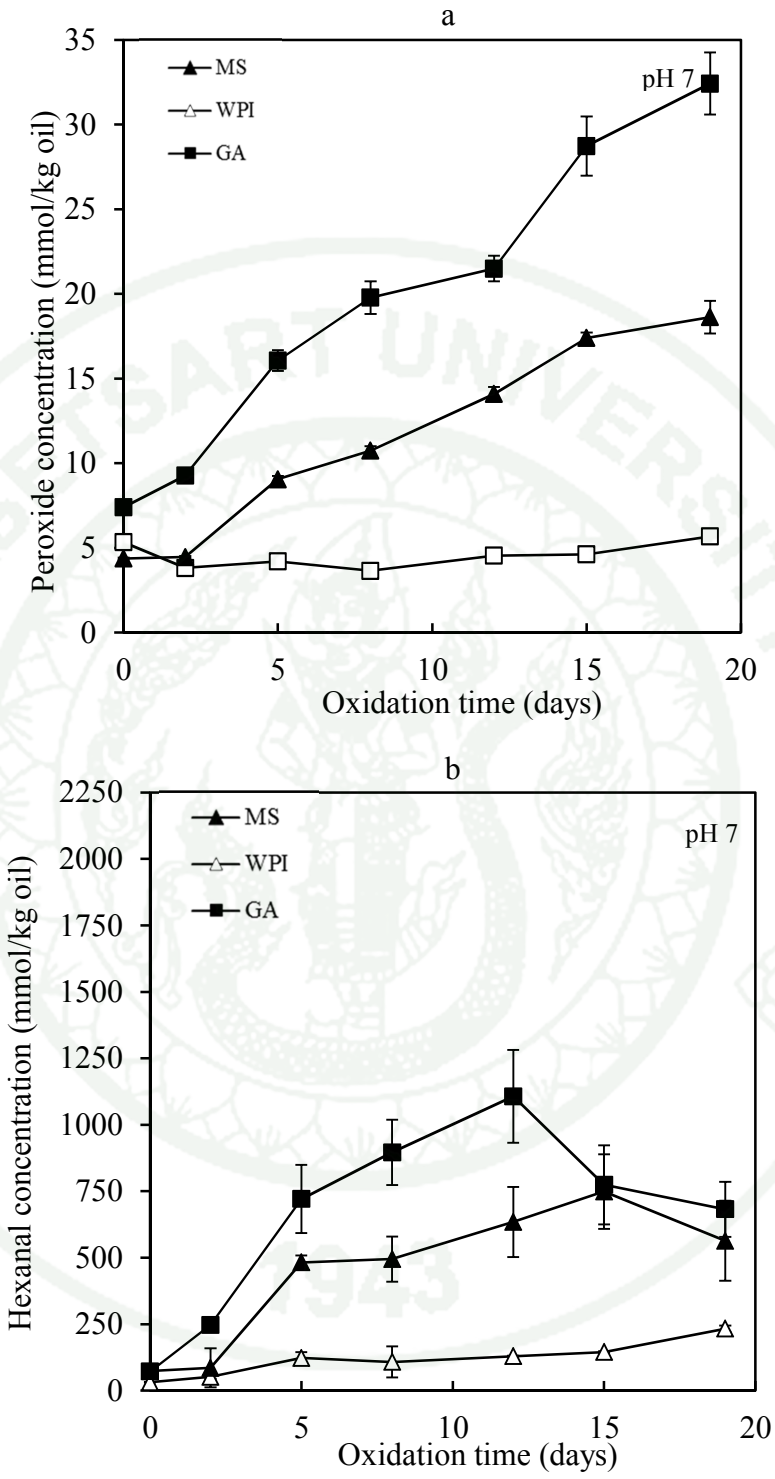


Figure 29 Formation of lipid hydroperoxide concentration (a) and hexanal concentration (b) in 5% rice bran oil–in–water emulsions stabilized by 0.45% whey protein isolate (WPI); 1.0% modified starch (MS) and 10.0% gum arabic (GA) at pH 7 during storage at 37 °C.

There appeared to be no correlation between droplet charge and the rate of lipid oxidation at pH 7. The magnitude of the negative charge on the lipid droplets increased in the following order: MS < GA < WPI (Figure 26), but the rate of lipid oxidation increased in the following order WPI < MS > GA (Figure 29). This result suggests that the relative affinity of cationic transition metals for the anionic surfaces of the lipid droplets did not play a major role in determining their oxidative stability. There are a number of physicochemical mechanisms that may contribute to the observed differences in the influence of biopolymer type on lipid oxidation rates. First, the specificity of ion–binding to biopolymers may influence the activity of pro–oxidant transition metals. Certain biopolymers bind cationic metal ions specifically due to the morphology of their binding sites (e.g., number, location, and direction of anionic groups), rather than through general non–specific electrostatic interactions. The strength of the binding of iron ions to biopolymer molecules may influence their pro–oxidant activity. Second, the composition and structure of the interfacial layer surrounding each of the lipid droplets is different in each system. Gum arabic and modified starch would be expected to form a relatively thick and porous interfacial layer, whereas WPI would be expected to form a relatively thin and dense interfacial layer. Third, the pro–oxidant may interact with biopolymers adsorbed to the oil–water interface or free within the aqueous phase. One would expected that interactions with adsorbed biopolymers may promote lipid oxidation by bringing the pro–oxidants into close proximity to the lipid substrate, whereas interactions with non–adsorbed biopolymers would inhibit lipid oxidation by keeping the pro–oxidants away from the lipid phase (Hu *et al.*, 2003a; Faraji *et al.*, 2004; Kellerby *et al.*, 2006a; Waraho *et al.*, 2011). The GA–stabilized emulsions had the highest level of biopolymer present, and therefore one would expect them to have the highest amount of non–adsorbed biopolymer. Nevertheless, this emulsion was the one that was most unstable to lipid oxidation. Finally, there may have been different levels of pro–oxidant impurities in the different biopolymer ingredients used. For example, many polysaccharide gums are known to contain relatively high levels of multivalent ions, such as transition metals (Katsuda *et al.*, 2008).

The results of the stablility of rice–bran–oil in water emulsion can be concluded that the emulsion formation using whey protein isolate or polysaccharide can produce the small droplets. The suitalbe concentration of each emulsifier was 0.45 %wt WPI, 1.0 %wt MS and 10.0 %wt GA, respectively. Emulsion formed using the MS and GA had much higher stability to environmental stresses (pH, salt, and thermal processing) while WPI had high stability under stresses at neutral and acidic condition. When considering lipid oxidative stability rice bran oil emulsion stabilized by MS and WPI were relatively stable to lipid oxidation, whereas those containing GA–stabilized droplets were highly unstable to the oxidation. This is because GA has the highest level of biopolymer present and consists of anionic polysaccharides and protein fractions mixture, which can bring the pro–oxidants into close proximity to the lipid substrate. Moreover, compared with those of WPI and MS, the larger particle of oil droplet emulsion stabilized by GA can lead to the oxidation degradation of the emulsion. Hence, in the next study, only MS and WPI were used as the emulsifiers for crude rice bran oil emulsion.

3. The formation, stability and physicochemical properties of encapsulated rice bran oil

As the results from previous study on the suitable rice bran oil extraction method, cold pressed Khao–dawk–mali 105 rice bran oil was used for the study in this section. The purpose of this study was to examine the influence of wall material on the emulsion formation, stability and physicochemical properties of encapsulated rice bran oil. Two different kinds of food grade biopolymers used as coating material in this study: whey protein isolate and modified starch were selected based on difference in their molecular characteristics with high stability under environmental stresses as shown in previous study.

3.1 Emulsion formation

Rice bran oil emulsions were prepared using similar condition as used in previous study (Charoen *et al.*, 2011, 2012). Homogenizing 5 wt% oil phase with 95% aqueous phase containing different emulsifier types and concentrations (0.02–10% WPI or 0.25–10% MS in 10.0 mM citrate buffer at pH 7) The dependence of the mean droplet diameter (d_{43}) of the resulting emulsions on initial emulsifier concentration was then measured 3 h after homogenization.

The mean droplet diameter tended to decrease as the emulsifier concentration was increased (Figure 30) which can be attributed to the fact that there was more emulsifier cover the newly formed oil–water interface created during homogenization.

The emulsifier concentration demarking Region I–II was around 1.74% for WPI and 3.5% for MS (Figure 30). The minimum droplet diameter that could be produced depended on emulsifier type and concentration, droplet diameter at 1.74 wt% of WPI d_{43} = 0.22 ± 0.002 μm and 3.5 wt% of MS d_{43} = 0.28 ± 0.01 μm. In the remainder of the experiments, rice bran oil emulsions was prepared using emulsifier concentrations that were capable of producing small droplet sizes without having too much excess emulsifier present: 1.74 wt% for WPI and 3.5 wt% for MS per 5.0% rice bran oil. A two–fold higher oil content was used to prepare the 10 wt% rice bran oil–in–water emulsions for spray drying, and therefore 3.5 wt% WPI and 7.0 wt% MS was used to prepare the powder.

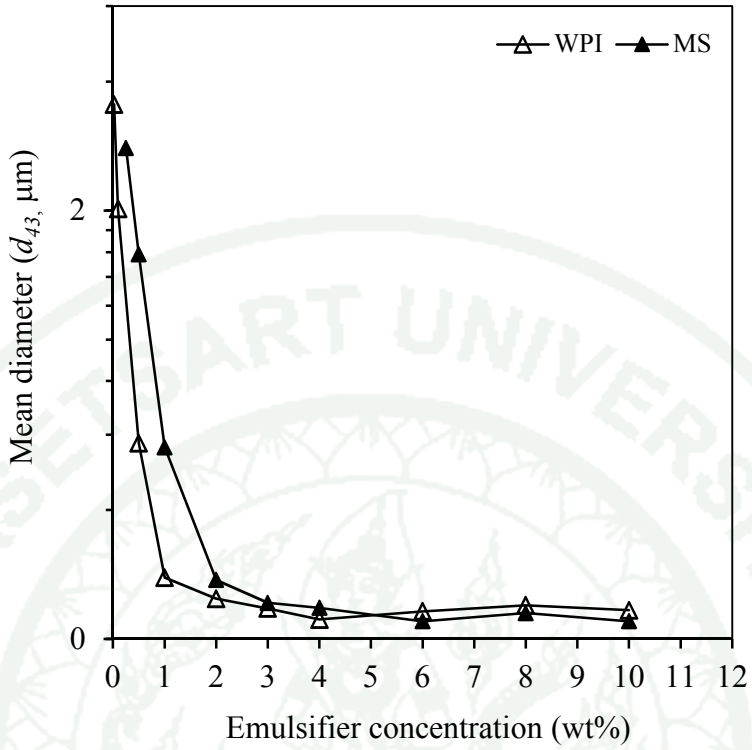


Figure 30 Emulsifier concentration dependence on the mean particle diameter of dilute 5% cold pressed rice bran oil–in–water emulsion stabilized by whey protein isolate (WPI) or modified starch (MS).

3.2 Physical properties of spray dried powder

Physical and reconstitution properties of spray–dried rice bran oil powder formed using either WPI or MS as emulsifiers are reported in Table 16. The powder contained 1.9 and 2.2% moisture and had water activities of 0.16 to 0.11 for WPI and MS, respectively. The lightness (L^*) of the rice bran oil powder stabilized by MS was higher than the powder stabilized by WPI, which suggests that the particles in the MS powder were more effective at scattering light. The yellowness (b^*) of the powder stabilized by MS was lower than the powder stabilized by WPI, which can be attributed to the fact that the color intensity usually decreases as the lightness increases (McClements, 2002). The mean particle sizes (d_{43}) of spray–dried powder were 15.6 ± 0.3 μm for WPI and 17.2 ± 0. 7 μm for MS. There was an appreciable

increase in the fraction of large particle sizes present in the powder stabilized by modified starch (Figure 31), suggesting that either droplet flocculation or coalescence had occurred before the powder dried. The fact that the powder contained relatively small particles (d_{43} < 25 μm) suggest that they could be very cohesive, which has been reported to lead to poor flowability (Jinapong *et al*., 2008; Masters, 1991).

The rationale behind this poor flowability at small particle sizes is due to the large surface area per unit mass of powder. Hence, there is more contact surface area between powder particles available for cohesive forces, in particular, and frictional forces to resist flow (Fitzpatrick *et al*., 2004; Fitzpatrick, 2005). Moreover, the rice bran oil powder obtained in this study contained approximately 30–32% fat (Table 16). This high fat content also caused the powder to have very poor flowability (Perez–Munoz and Flores, 1997; Fitzpatrick *et al*., 2004). The spray–dried powder obtained from small scale spray dryers often have a small particle size <50 μm, with poor handling and reconstitution properties (Master, 1991). These powder require agglomeration in order to improve handling and reconstitution properties (Fuchs *et al*., 2006; Turchiuli *et al*., 2005).

Table 16 The physical properties of spray–dried rice bran oil powder stabilized by different biopolymer.

Powder characteristics	Biopolymer	
	Whey protein isolate (WPI)	Modified starch (MS)
- Moisture content	2.16±0.22[a]	1.93±0.18[b]
- a_w	0.161±0.02[a]	0.107±0.01[b]
- Color :		
L* value	91.446±0.67[b]	94.003±1.26[a]
a* value	0.107±0.081[b]	0.173±0.02[a]
b* value	15.633±0.565[a]	9.803±0.196[b]
- Particle size (μm)	15.64±0.33[a]	17.23±0.67[a]
- Density :		
bulk density	0.277±0.01[b]	0.373±0.02[a]
tapped density	0.438±0.004[b]	0.508±0.02[a]
- Carr index (*CI*)	36.83±2.36[a]	26.67±1.53[b]
- Hausner ratio (*HR*)	1.584±0.06[a]	1.364±0.03[b]
- Wettability (time: s)	87.0±12.0[a]	72.0±8.6[b]
- %Dispersibility	69.47± 2.58[a]	71.05± 2.16[a]
- % Total fat	32.69±2.86[a]	30.26±1.37[b]
- % Free fat	1.57±0.04[b]	2.24±0.13[a]
- Encapsulation efficiency (%EE)	95.19±0.31[a]	92.58±0.49[b]

[a]Mean ± standard deviation of duplicate measurements. Mean values with different letters were significantly different ($P \leq 0.05$).

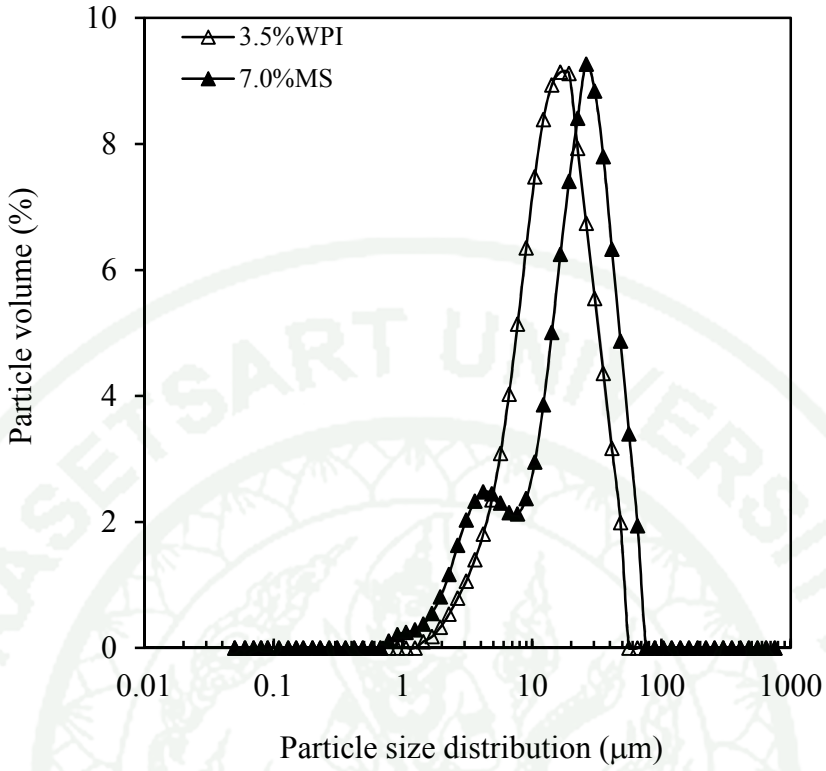


Figure 31 Representative particle size distributions of rice bran oil dried powder stabilized by (a) 3.5% whey protein isolate (WPI) and (b) 7.0% modified starch (MS).

A dry product with high bulk density can be packed more efficiently compared to a product with low bulk density. The Hausner ratio (*HR*) and the Carr index (*CI*) are typically used to characterize powder properties (Barbosa–Canovas and Juliano, 2005). The results showed that the powder stabilized by MS had fair flowability and intermediate cohesiveness as classified by the Carr index and Hausner ratio (Table 16). On the other hand, the powder stabilized by WPI had poorer flow characteristics as evidenced by their higher *CI* and *HR* values which may have been due to their smaller particle size and the presence of many fine particles (Jinapong *et al*., 2008; Masters, 1991). The poor flowability of small particles has been attributed to the large surface area per unit mass of powder. Hence, there is more contact surface area between powder particles available for cohesive forces, in particular, and frictional forces to resist flow (Fitzpatrick *et al.*, 2004). There was no significant

difference between the %dispersibility of the powders stabilized by WPI and MS ($P \leq 0.05$).

Encapsulation efficiencies refer to the potential of the wall material to encapsulate or hold the core material inside the microcapsule. Encapsulation is also related to the shelf life of oil content in the powder (Zhang *et al.*, 2007; Idham *et al.*, 2011). The results from the total oil and free oil were calculate followed above equation for encapsulation efficiency (%EE), and also showed in Table 16. The spray dried powder stabilized by WPI had higher encapsulation efficiencies (95.19%) than MS (92.58%). This result indicates that both WPI and MS lead to the formation of powder that retain a high percentage of the oil within the wall material.

The effectiveness of interactions depended on the chemical and physical structure of the material. Those material differ in molecular characteristics: whey protein isolate is a mixture of amphoteric globular proteins (Dalgleish, 1997; Wilde, 2000); modified starch consists of starch molecules that have been chemically reacted with octenyl succinic anhydride (OSA) to give them some hydrophobic character (Tan, 2004; Trubiano, 1995; Given, 2009). As a result, each type of biopolymer emulsifier has different abilities to form and stabilize emulsions. During homogenization, globular proteins tend to adsorb more rapidly to oil droplet surfaces than polysaccharides and hence are capable of forming smaller droplets (McClements, 2005). Furthermore, Barbosa *et al*. (2005) reported that the addition of maltodextrin as emulsifier had ability to encapsulate higher amount of bixin. As mentioned in Shahidi *et al.*'s study (2004) they found that phenolics compound and flavonols may form complexes with polysaccharides and the affinity of compound to polysaccharides depends on the water solubility, molecular size, conformational mobility and shape of bioactive compound.

The scanning electron micrographs of spray–dried powder stabilized by WPI and MS are shown in Figure 32. The powder had particles of various sizes, which agree with the results obtained by light scattering. Most of the particles were spheroid in shape with relatively smooth surfaces, but there was evidence of fissures

or cracks in some particles. These cracks may allow gasses to penetrate into the core material and promote oxidative instability. The formation of non–uniform surfaces may have occurred during the dehydration step in spray drying leading to rapid shrinkage. Similar morphologies have been reported in powder formed during microencapsulation of monoterpenes and ascorbic acid using gum arabic as a wall material (Bertolini *et al.*, 2001; I Ré, 1998).

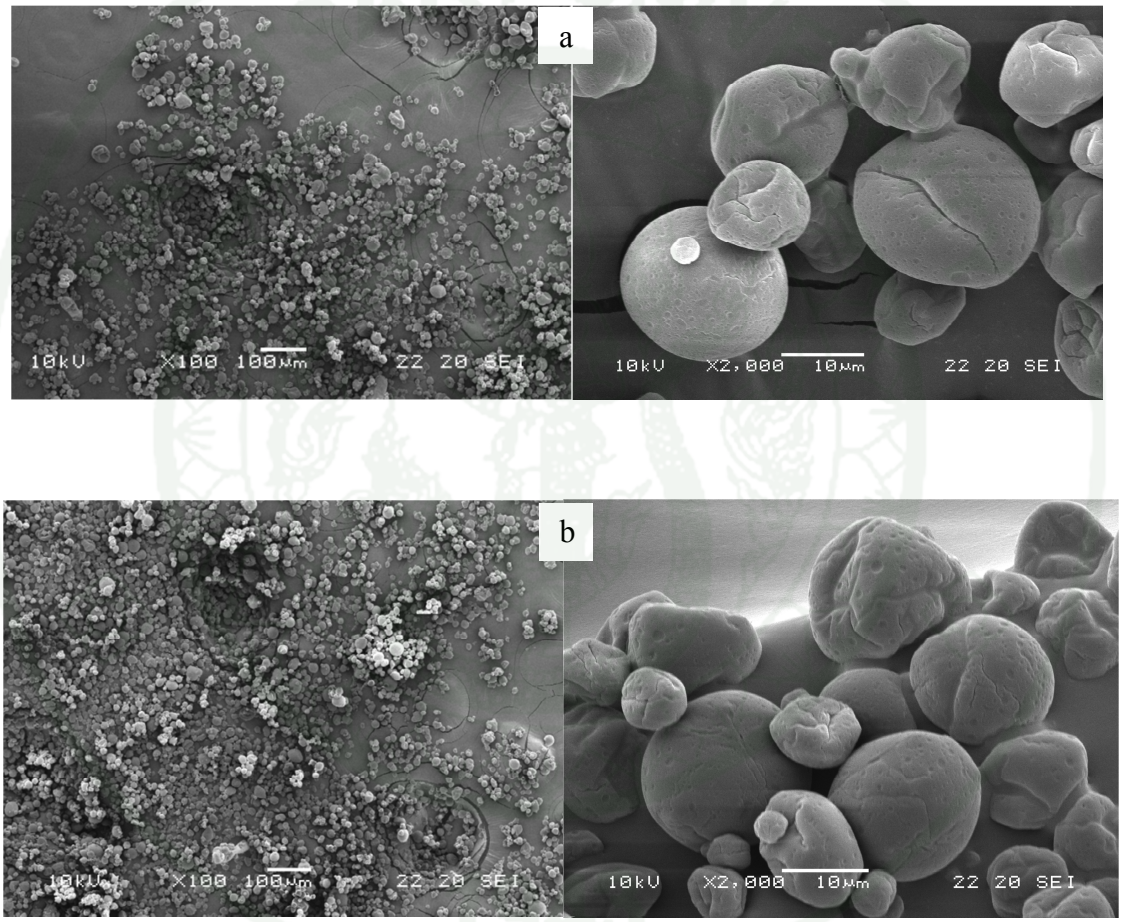


Figure 32 Scanning electron micrographs of spray–dried powder stabilized by (a) whey protein isolate (WPI) 3.5 wt% and (b) modified starch (MS) 7.0 wt%.

3.3 Identification of phytosterol in rice bran oil powder

Phytosterols are important lipophilic ingredients with reported health benefits, so identification of phytosterols was made to all encapsulated samples to confirm the presence of phytosterols by comparing it with the stanyl fatty acid ester mixture. The HPLC chromatogram data (Figure 33) showed all the spray–dried samples that contained phytosterol or sitosterol, campesterol, stigmasterol or els. From the chromatogram of sample where the retention time of β–sitosterol, campesterol and stigmasterol was 23.023, 21.743, 23.189 for WPI and 22.987, 21.727, 23.168 for MS, respectively. Although the retention time for samples and standard was slightly different, the peaks were clearly identified as β–sitosterol, campesterol and stigmasterol. Interestingly, the identification of total phytosterol in crude oil encapsulated by spray drying technique in order to reduce the deterioration of bioactive compound from the environment factors (such as temperature, light, moisture and oxygen). The results indicated that the total phytosterols content of rice bran oil powder stabilized by WPI contained 13,650.6 ppm, while powder stabilized by MS contained 15,885.3 ppm.

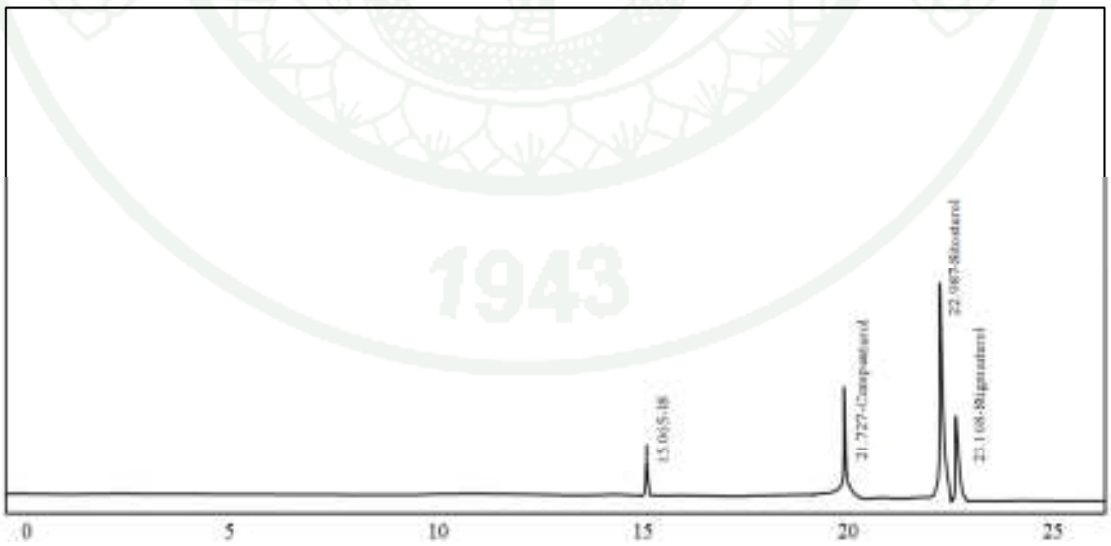


Figure 33 GC–FID Chromatogram of phytosterols (sito– camp– stigma– etc.) in rice bran oil encapsulated powder stabilized by modified starch (MS).

3.4 Sorption isotherm study

The purpose of this study was to examine the influence of wall material composition on the formation, stability and physicochemical properties of encapsulated rice bran oil. There were two different kinds of food grade biopolymer as coating–materials in these studies; whey protein isolate (WPI) and modified starch (MS) which used to formed and encapsulated rice bran oil into the powder form.

Water sorption properties of dried powder are governed by the composition of nonfat solutes (carbohydrate, proteins, minerals, and other minor components) which often exist in an amorphous state in spray dried powder (Roos, 1995). In the present study, whey protein and/or modified starch were used to produce the powder; the total fat contents of the powder varied from 30.3–32.7%. Steady–state water contents has been reached after 9 days storage, and these values were used to fit to the BET and GAB models as equation 1–2 followed.

Experimental water sorption isotherms for rice bran oil powders stabilized by WPI and MS are reported in Figure 34. The BET and GAB isotherm models were fitted to the data and the parameters are presented in table 17. Both BET and GAB models showed a good fit to the experimental data, with high R^2 values, RSME and mean relative deviation modulus (E). The sorption isotherms fitted to the BET model are presented in Figure 34a and to the GAB model in Figure 34b.

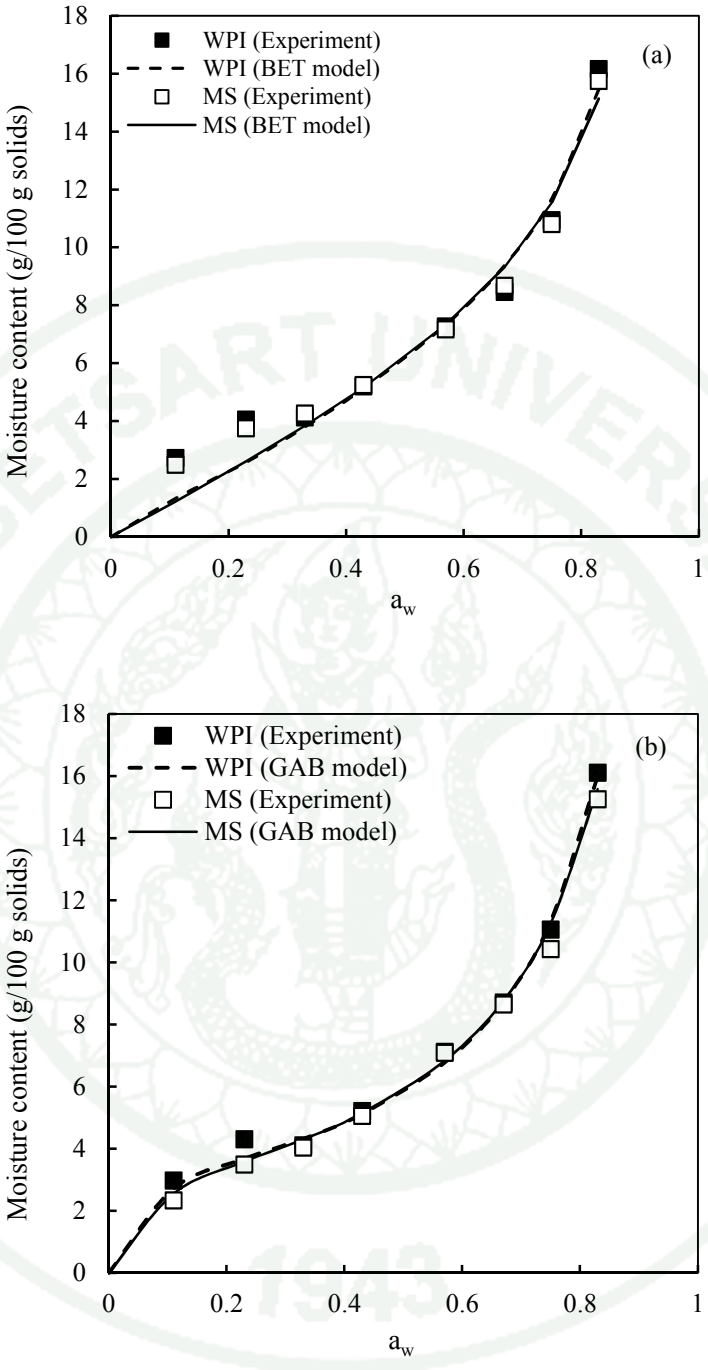


Figure 34 Sorption isotherm of spray dried rice bran oil stabilized by whey protein isolate (WPI) and modified starch (MS) obtained for different sorption model (a) BET model (b) GAB model.

Table 17 Estimated BET and GAB parameters for rice bran oil powder stabilized with different carrier agent.

Models	Parameters				R^2	E (%)	RSME
	X_m	C_{BET}	C_{GAB}	K_{GAB}			
BET							
WPI	7.0790	0.1514	–	–	0.9507	15.5362	0.9467
MS	7.8364	0.1387	–	–	0.9628	14.4562	0.8105
GAB							
WPI	3.0558	–	47.5834	0.9757	0.9922	5.3085	0.3767
MS	3.1973	–	24.1440	0.9601	0.9932	4.0772	0.3470

Both BET and GAB models are based on the assumption that a monolayer of water forms on the surface of the material. The monolayer moisture content (X_m) indicates the amount of water that is strongly adsorbed to specific sites at the food surface and is considered as an important value to assure food stability (Righetto and Netto, 2005; Moraga *et al*., 2006). The X_m values obtained for spray–dried powder stabilized by WPI or MS varied from 7.08 to 7.84 g H_2O/100 g solids for the BET model and from 3.06 to 3.20 g H_2O/100 g solids for the GAB model. The powder stabilized by MS had a great amount of water adsorption than the one stabilized by WPI. The differences in water adsorption may be explained by the differences in the chemical structure of each carrier. Modified starch consists of carbohydrate molecules that have a great number of hydrophilic groups, although some of molecules have been chemically reacted with octenyl succinic anhydride (OSA) (Trubiano, 1995; Tan, 2004; Given, 2009). Whey protein isolate is a mixture of amphoteric globular proteins (Galgleish, 1997; Wilde, 2000).

Changes in the physical characteristics of powder stored at different relative humidity (RH) could be observed (25 ºC). When stored at RH of 43% or lower, the powder were free–flowing powder for both carrier types used. At RH above 65%, the powders were hard and sticky and appeared more yellowish. The

influence of water activity on the equilibrium moisture content of rice bran oil powder stabilized by the different emulsifiers is shown in Table 18.

Table 18 Equilibrium moisture content of powder stabilized with different emulsifier.

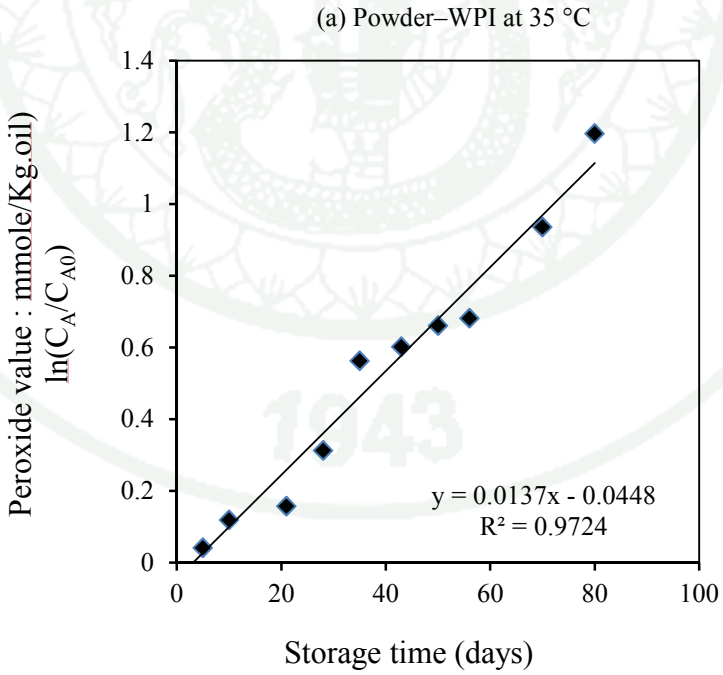
a_w	Equilibrium moisture content, X_e (g/100g dry matter)	
	Whey protein isolate	Modified starch
0.11	2.7211±0.1590	2.4909±0.1347
0.23	3.5031±0.2260	3.7502±0.0912
0.33	4.1263±0.0323	4.2640±0.2141
0.43	5.1983±0.0116	5.2443±0.1676
0.57	7.273±0.1567	7.1718±0.2194
0.67	8.4642±0.2403	8.6769±0.2458
0.75	10.9362±0.1026	10.8037±0.1682
0.86	16.1694±0.1576	15.7528±0.1527

The properties and stability of amorphous powdered foods has been related to their physical state (glassy *versus* rubbery). The transition from one state to another normally occurs over a range of temperatures that can be characterized by a glass transition temperature (T_g) (Roos and Karel, 1991b; Sá and Sereno, 1994). When the temperature increases above T_g, there are changes in the properties of amorphous solids: increase in free volume; increase in specific heat; decrease in viscosity; changes in physical properties such as collapse, loss of shape, shrinkage or stickiness (Schebor *et al*., 1999; Moraga *et al*., 2004). The state of the system (glassy *versus* rubbery) also determines the rate of chemical reactions, such as oxidation.

3.5 Kinetic analysis of dried powder

Most reactions that showed loss in food quality may be described by zero or first order (Pua *et al*., 2008). In order to determine the reaction order of a quality attribute, the experimental data obtained was fitted to linear equation which

mentioned as equation 11–13. The reaction of lipid oxidation (lipid hydroperoxide) measured at different storage times versus time was plotted and data obtained was fit by a linear equation. If the data showed a good fit to a linear equation, then the data indicated a zero order reaction. On the other hand, if the $\ln[C_t–C_0]$ versus time fits a linear equation, then the reaction was considered to be a first order reaction (Singh, 2000). Figure 35a shows a linear relationship (R^2=0.9724) between [$\ln C_t/C_0$] and time for rice bran oil encapsulated in different wall materials at 35 ºC. These results suggest that the degradation of lipid hydroperoxide in powder stabilized by WPI followed first order reaction kinetics. On the other hand, powder stabilized by MS showed a linear relationship (R^2=0.9359) between $C_a–C_{a0}$ *versus* time, suggesting that lipid degradation was better described as zero order for this system (Figure 35b). According to Singh (2000), the zero order rate was useful in describing reactions like enzymatic degradation, non–enzymic browning and lipid oxidation that led to development of rancid flavors.



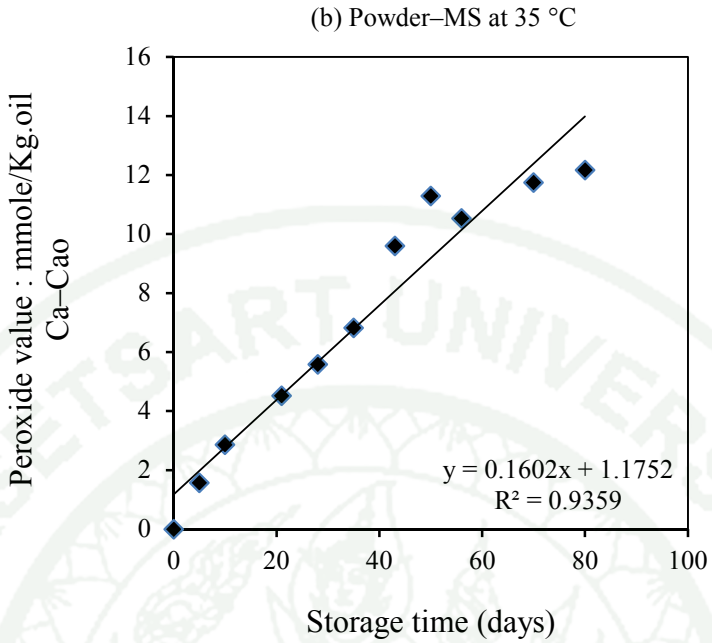


Figure 35 Kinetic reaction order for lipid degradation in dried powder with different carriers (a) first order of dried powder, (b) zero order of dried powder.

The rate constants (k) obtained from the plots of lipid degradation versus time are shown in Table 20. The results show that the effect of storage temperature on the degradation kinetics for lipid hydroperoxide in encapsulated powder were significantly different ($P \leq 0.05$) for the two biopolymer emulsifiers. An increase in storage temperature led to an increase in the rate constant (k) of all powder with WPI and MS. The degradation rate constants of the WPI–powder (0.010 to 0.023 per day) were lower than the MS–powder (0.136–0.268 per day). The degradation rated for the MS–powder was significantly higher ($P \leq 0.05$) the WPI–powder at all storage temperatures studied. Furthermore, when activated energy (Ea) of lipid degradation of dried powder was considered, it was found that lipid degradation of oil in dried powder stabilized by WPI had higher Ea than that of MS. As a result, there were some lipid hydroperoxide delivertives found in the product.

This result may be attributed to the fact that whey protein isolate gave the highest encapsulation efficiencies (EE) when used as a wall material. The high encapsulation efficiencies and low degradation rates indicated the stability and prolonged shelf life of the lipid oxidation in the dried powder.

Table 19 Degradation parameter of lipid oxidation in rice bran oil encapsulated powder with different stabilizers in various storage temperature.

Carriers	temperature (°C)	R^2		
		n=0	n=1	n=2
		$C_A = -kt + C_{A0}$	$\ln (C_A/C_{A0}) = -kt$	$1/C_A = kt+1/C_{A0}$
WPI	25	0.9157	0.9194	0.8931
	35	0.9179	0.9724	0.9642
	45	0.9265	0.9748	0.9343
MS	25	0.9394	0.8825	0.7690
	35	0.9359	0.8680	0.7339
	45	0.9546	0.8823	0.7758

Table 20 Estimate kinetic reaction constant value for dried powder with different stabilizers.

Temperature (°C)	kinetic reaction constant	
	Whey protein isolate; n=1	Modified starch; n=0
25 °C	0.0103	0.1362
35 °C	0.0137	0.1602
45 °C	0.0225	0.2680
Ea/R	3690.2	3187.9
Ea (J/mole)	30,680	26,504.2

3.6 Color change during storage

The color of powder during storage is an important quality attribute. The initial color parameters (L*, a*, b* values) for encapsulated rice bran oil were: 91. 5 ± 0.7, 0.11 ± 0.08 and 15.6 ± 0.7 for WPI–powder; 94.0 ± 1.3, 0.17 ± 0.02 and 9.8 ± 0.2 for MS–powder. The initial values showed that the MS as encapsulation agent gave high lightness value compared with the WPI agent. For the yellow color, WPI gave higher yellow (b*value) color than MS. (Table 21). showed that the encapsulating agent significantly affected the color change. On the other hand the color showed no difference between storage period and temperature. According to Table 21, the change of a_w of encapsulated oil stabilized WPI and MS, visually observed in all spray–dried powder and storage temperature used as verified by the similar fluctuated ($P > 0.05$). It seems to be that the powder storage in 45 ºC was increase after 58 days.

Table 21 The change of color and a_w during storage.

Temperature (ºC)	Storage time (day)	Whey protein isolate				Modified starch			
		L*	a*	b*	a_w	L*	a*	b*	a_w
25	0	86.8±0.01	–1.43±0.02	16.9±0.04	0.16±0.00	91.7±0.01	–0.31±0.02	9.32±0.01	0.10±0.00
	7	84.9±0.02	–0.73±0.01	19.0±0.01	0.20±0.01	89.9±0.01	–0.11±0.02	11.8±0.04	0.13±0.00
	14	84.0±0.09	–0.82±0.01	19.3±0.06	0.21±0.00	89.9±0.57	–0.25±0.11	11.1±0.92	0.12±0.01
	22	85.0±0.02	–1.04±0.01	18.4±0.01	0.17±0.00	90.2±0.29	–0.17±0.02	10.9±0.52	0.15±0.00
	30	84.7±0.71	–1.2±0.06	18.8±0.45	0.19±0.00	90.0±0.37	–0.25±0.08	10.5±0.16	0.11±0.00
	37	83.5±0.34	–1.0±0.07	18.9±0.03	0.21±0.00	89.5±0.01	–0.28±0.08	11.3±0.37	0.12±0.00
	52	85.3±0.03	–1.01±0.03	18.6±0.17	0.18±0.01	90.1±0.66	–0.13±0.02	10.6±0.63	0.13±0.01
	58	85.4±0.01	–1.38±0.05	17.2±0.03	0.19±0.00	90.5±0.02	–0.55±0.01	10.1±0.02	0.15±0.00
	72	83.9±0.04	–1.06±0.07	18.6±0.00	0.23±0.00	90.1±0.02	–0.36±0.01	10.6±0.03	0.12±0.00
35	0	86.8±0.82	–1.43±0.03	16.9±0.02	0.16±0.00	91.7±0.02	–0.31±0.00	9.32±0.02	0.10±0.01
	7	85.6±0.11	–1.09±0.06	17.1±0.03	0.18±0.00	89.2±0.02	–0.04±0.01	12.1±0.04	0.15±0.0
	14	85.5±0.18	–1.20±0.03	16.6±0.07	0.19±0.00	90.1±0.18	–0.32±0.03	10.9±0.07	0.16±0.01
	22	84.9±0.08	–1.04±0.11	18.4±0.13	0.18±0.01	90.5±0.01	–0.29±0.01	10.3±0.01	0.16±0.01
	30	85.0±0.04	–1.3±0.01	17.7±0.21	0.18±0.00	90.1±0.08	–0.36±0.01	10.7±0.09	0.15±0.02
	37	84.7±0.03	–1.3±0.02	17.8±0.05	0.17±0.00	89.8±0.01	–0.32±0.00	10.7±0.02	0.14±0.00

Table 21 (Continued)

Temperature (ºC)	Storage time (day)	Whey protein isolate				Modified starch			
		L*	a*	b*	a_w	L*	a*	b*	a_w
35	52	84.8±0.01	–1.14±0.03	18.0±0.38	0.18±0.01	90.1±0.33	–0.13±0.01	10.8±0.27	0.15±0.00
	58	84.1±0.04	–1.32±0.02	17.5±0.07	0.18±0.00	89.5±0.16	–0.34±0.58	11.1±0.45	0.17±0.01
	72	84.8±0.01	–1.25±0.03	17.2±0.02	0.22±0.01	90.0±0.01	–0.51±0.01	10.6±0.02	0.21±0.00
45	0	86.8±0.01	–1.43±0.04	16.9±0.02	0.16±0.02	91.7±0.01	–0.31±0.02	9.32±0.03	0.10±0.02
	7	86.2±0.06	–1.11±0.08	16.4±0.01	0.23±0.00	89.5±0.01	–0.22±0.01	11.0±0.04	0.15±0.00
	14	85.7±0.23	–1.12±0.03	16.7±0.25	0.23±0.01	89.9±0.04	–0.23±0.00	11.3±0.03	0.14±0.02
	22	84.8±0.16	–1.09±0.58	17.5±0.45	0.21±0.03	90.2±0.00	–0.4±0.04	10.9±0.01	0.15±0.01
	30	85.9±0.03	–1.11±0.04	16.8±0.01	0.22±0.00	90.0±0.12	–0.35±0.06	10.5±0.76	0.12±0.02
	37	84.6±0.57	–1.02±0.09	17.2±0.78	0.21±0.00	89.8±0.03	–0.35±0.02	10.3±0.05	0.14±0.01
	52	84.7±0.73	–0.67±0.06	17.8±0.66	0.20±0.00	90.5±0.16	–0.34±0.06	10.3±0.11	0.14±0.00
	58	84.3±0.46	–1.01±0.23	17.4±0.13	0.20±0.00	90.5±0.11	–0.54±0.02	10.1±0.08	0.14±0.00
	72	84.6±0.80	–0.8±0.01	17.7±0.67	0.23±0.02	90.4±0.03	–0.65±0.00	9.16±0.05	0.20±0.01

This study can summarized that WPI and MS had high efficiency to encapsulate rice bran oil (92–95 %EE). The 3.5 wt% of WPI and 7.0 wt% of MS led to the formation of small particle droplet (<300 nm), resulting in very small size of spray–dried powder (<25 μm). However, handling and reconstitution properties of the powder, i.e. flowability and wettability were poor due to their very small size dried particle. The results of the sorption characteristics of powder stabilized by WPI or MS showed that GAB model was suitable for describing the relationship between the equilibrium moisture content and GAB also can be used to calculate the monolayer moisture content. The kinetics of lipid degradation of rice bran oil dried powder stabilized by WPI or MS were determined as a function of temperature. The results showed the first order reaction described for lipid oxidation degradation powder stabilized WPI while zero order reaction described for powder stabilized MS.

4. Consumer survey and powder application in food model

4.1 Consumer demography

The marketing survey results in term of needs of 213 consumers consist of three sections. The first is the general information of the consumer. The second is the consumer's awareness on health, disease, dietary guidance, rice bran product buying decision, and the third section is the consumer behavior showed as following.

According to the survey, consumers were asked about the general consumer's information including gender, age, education, occupation and the average income per month. The results were shown in Table 22. The results of the health awareness here were consistent with the results of a survey of health in the United States (Natural Marketing Institute's, 2007), where the top three health problems were problem of the heart (29%), problem of obesity (29%) and problem of the digestive tract (25%) (Table 23).

Table 22 The general consumer's information (N=213).

General information of consumer		Percentage (%)
Gender	Male	28.6
	Female	71.4
Years	<21	8.9
	21–30	35.2
	31–40	25.4
	41–50	17.8
	>50	12.7
Education	Primary school	0.9
	High school	5.6
	Diploma	5.2
	Bachelor degree	65.3
	>Bachelor degree	23.0
Occupation	None	2.8
	Business	5.2
	Employees	31.0
	Officially government	29.1
	Employer of the state	30.5
	etc.	1.4
Income per month	<10,000	12.5
(Baht)	10,001–15,000	32.0
	15001–20,000	25.0
	20,001–25,000	11.5
	25,001–23,000	15.0
	>30,000	4.0
Illness	Yes	20.2
	No	79.8

Table 23 The consumer's health awareness (N=213).

Health problem	Consumer; N=213 (%)	Range of important
Heart disease	174 (81.7)	1
Hypertension	155 (72.4)	2
Obesity	145 (68.1)	3
Arthritis	130 (61.0)	4
Diabetes	126 (59.2)	5
Digestive tract	124 (58.2)	6
Optical problems	112 (52.6)	7
Seasonal allergies	99 (46.5)	8

4.2 Consumer's behavior

4.2.1 The consumer's behavior

For consumer eating habits, the data from the consumer survey showed that 69.9% of consumers had three times meals and 46.6% of consumers bought raw ingredients. The consumer favorite method for cooking food was boil or scald (39.8%). The top five popular beverage products were vegetable and fruit (58.7%), soybean milk (42.3%), fresh milk (36.6%), coffee (32.9%) and carbonate water (31.5%).

The consumer survey on the consumption habits in term of the consumption frequency of products containing rice bran oil components showed that more than half of consumers (54.9%) consumed the product through the cooking oil, refined rice bran, while 34.7% and 33.3% of consumers consumed products containing rice bran oil as an ingredient in food, and as supplement tablets, respectively. The top three places where consumers buy these products, were department stores (50.2%), supermarkets (36.6%), and health products shop (16.9%). The value range of rice bran oil products that consumers can buy at a time were 51–100 baht (37.6%) and 101–200 baht (21.1%). In addition, 61.0% of the consumers

purchased by themselves and there was only 23.0% for children and relatives buying for their families.

4.2.2 The important factors for the consumer health problem avoidance

The levels of importance of twelve variables affecting the consumer health problem avoidance were rated by consumers, and reported as the mean score of each variable. The data was analyzed by factor analysis (FA) to group all variable into the new components.

As can be seen from Table 24 showing the levels of importance and the mean score of each variable, there were 6 factors with very important level such as main meals, food supplement, relax and sleep tight, exercise, mind peace and non–smoking. The levels of importance of the other 6 factors such as spice and herb, hobby etc. were moderate important.

The factor analysis techniques were used to reduce the number of variables to new component which had a high relative. For the results shown in Table 25 and Figure 36, three new components could be defined as “food consumption”, “recreational activities” and “health activities” including considered eigen value, variance explanation (%) and accumulated various explanation (%). This factor analysis results can explain 59.21% of the variation.

Table 24 Important factors affecting the consumer health problem avoidance.

Variable	Mean±S.D.*	Level of importance*
1. Main meals	4.02±0.8	Very important
2. Food supplement	3.55±0.9	Very important
3. Spice and herb	2.83±1.0	Moderate important
4. Relaxation and sleep tight	3.86±0.9	Very important
5. Exercise	3.55±1.0	Very important
6. Hobby	3.37±0.9	Moderate important
7. Mild peace	3.62±0.9	Very important
8. Travel to the countryside	3.08±0.9	Moderate important
9. Religion activity	2.92±0.9	Moderate important
10. House work	3.18±0.9	Moderate important
11. Non smoking	4.00±1.2	Very important
12. Annual health check	3.43±1.0	Moderate important

*The score is rated from 1 to 5 (1= less important; 5=extremely important)

Mean score 1.00–1.49 = very slightly important

Mean score 1.50–2.49 = slightly important

Mean score 2.50–3.49 = moderately important

Mean score 3.50–4.49 = very important

Mean score 4.50–5.00 = extremely important

Table 25 Factor loading of rotated component matrix on the consumer health problem avoidance practice.

Variable	Code	Factor and factor loading of variables		
		1*	2*	3*
Exercise	Exer	**0.704**	0.405	0.102
Hobby	Hobb	**0.567**	0.314	0.313
Mild peace	Mild	**0.662**	0.416	0.060
Travel to the countryside	Trav	**0.590**	0.310	0.244
Religion activities	Relig	**0.794**	–0.104	0.089
House works	House	**0.672**	0.094	0.030
Main meals	Meal	0.198	**0.620**	0.199
Non smoking	Nons	–0.017	**0.791**	–0.067
Annual health check	Annu	0.398	**0.509**	0.055
Relax and sleep tight	Relax	0.536	**0.575**	0.055
Food supplement	Suppl	0.058	0.062	**0.901**
Spice and herb	Spice	0.171	0.041	**0.845**
Eigen value		4.533	1.476	1.026
Variance explained (%)		37.33	13.30	8.58
Accumulated explained (%)		37.77	50.63	59.21

*Factor loading in bold are considered significant variables and highly related to the same component.

4.2.3 Factors affecting the consumer buying decision

The levels of importance of the factors affecting consumer decision on buying rice bran or rice bran oil products were shown in Table 26. The factors with high level of importance were taste, nutritional value, properties to prevent or treat the disease, clean and safe, price and the convenience of buying. On the other hand, the level of importance of the other factors such as the product appearance, usage

preparation, brand of product, packaging, modern and beauty of package, packing size and advertisement were at moderate importance.

Table 26 Important factors influencing consumer's satisfaction regarding rice bran and rice bran oil products.

Variable	Mean±S.D.*	Level of importance*
1. Product appearance	3.31±0.9	Moderate important
2. Tastes	3.51±0.9	Very important
3. Nutritional value	4.12±0.8	Very important
4. Product properties to prevent or treat disease	4.00±0.9	Very important
5. The preparation before use	3.48±0.9	Moderate important
6. Clean and Safe	4.19±0.8	Very important
7. Brand of product	3.30±0.9	Moderate important
8. Modern and beauty of packaging	2.91±0.8	Moderate important
9. Packing size	3.15±0.8	Moderate important
10. Price	3.64±0.8	Very important
11. Convenience of buying	3.73±0.8	Very important
12. Advertisement	3.04±0.8	Moderate important

*The score is rated from 1 to 5 (1= less important; 5=extremely important)

Mean score 1.00–1.49 = very slightly important

Mean score 1.50–2.49 = slightly important

Mean score 2.50–3.49 = moderately important

Mean score 3.50–4.49 = very important

Mean score 4.50–5.00 = extremely important

Mean score 3.50–4.49 = very important

Mean score 4.50–5.00 = extremely important

For the results shown in Table 27 and Figure 36, the factor analysis techniques were used to reduce the number of variables and two new components could be defined as “product properties aspect” and “marketing aspect”.

Table 27 Factor loading of rotated component matrix on consumer buying decision for rice bran and rice bran oil products.

Variable	Code	Factors and Factor loading of variables	
		1*	2*
1. Tastes	Taste	**0.520**	0.398
2. Nutritional value	Nutri	**0.838**	0.037
3. Product properties to prevent or treat disease	Prev	**0.811**	0.092
4. The preparation before use	Use	**0.641**	0.315
5. Clean and Safe	Safe	**0.773**	0.149
6. Price	Price	**0.527**	0.117
7. Convenience of buying	Conv	**0.538**	0.364
8. Product appearance	App	0.337	**0.572**
9. Brand of product	Brand	0.225	**0.651**
10. Modern and beauty of packaging	Modern	0.065	**0.871**
11. Packing size	Size	0.210	**0.735**
12. Advertisement	Add	0.047	**0.725**
Eigen value		4.701	1.730
Variance explained (%)		39.17	15.41
Accumulated explained (%)		39.17	54.58

*Factor loading in bold are considered significant variables and highly related to the same component.

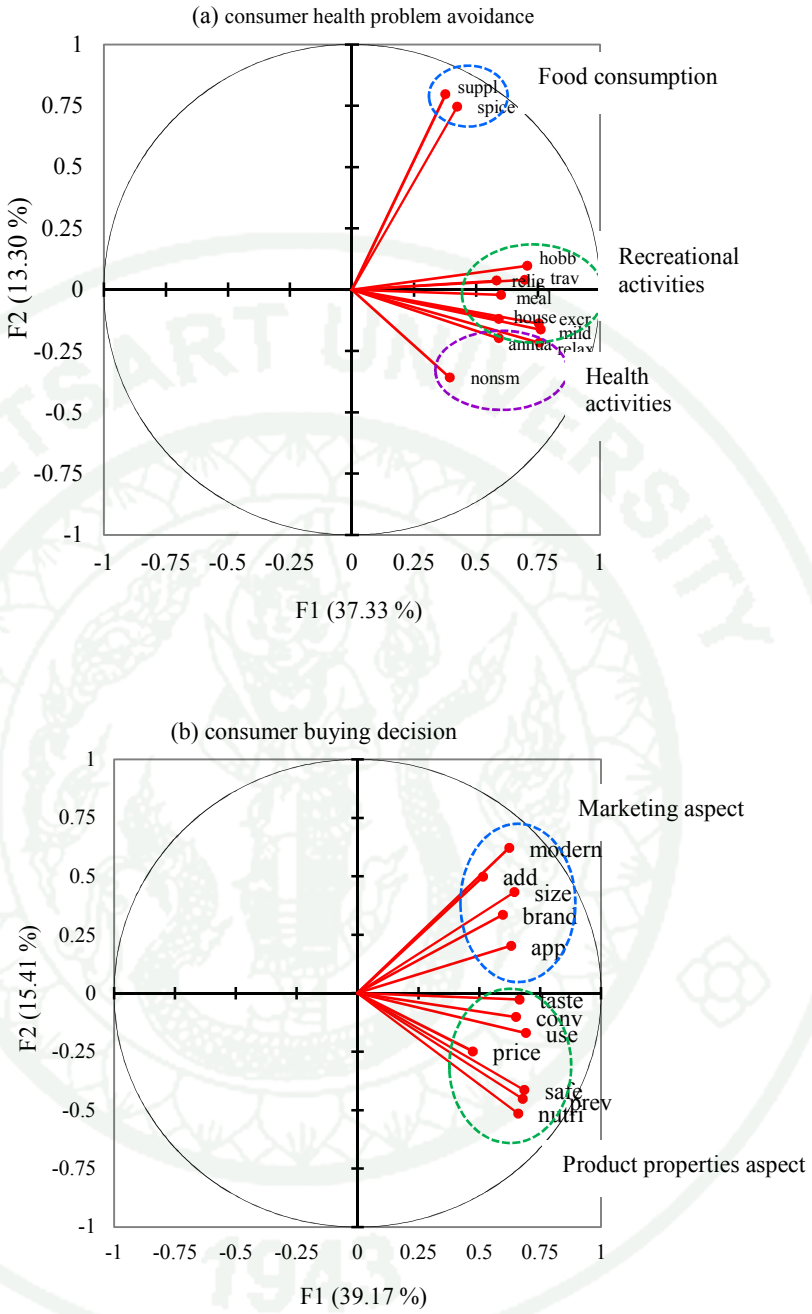


Figure 36 A biplot of the factor loading of variable; (a) consumer health problem avoidance and (b) consumer buying decision.

The general market survey about the need and behavior of customers including the information of disease awareness, the behavior of buying, eating and expecting in new products can be concluded that the consumers have been aware of diseases such

as heart diseases as well as blood circulation system. In addition, consumers have to prepare themselves in good condition in order to protect all those diseases by eating healthy food, having enough relaxation with less stress and avoiding getting involved in all kinds of smoking and alcoholic drinks. The conclusion from the behavior of having products contained rice bran oil reveals that consumers are not familiar with the new products which are not variously available in the market. As a matter of fact, new products of rice bran oil with suitable product properties aspect such as taste, nutrition value, product properties to prevent or treat disease etc. and the marketing aspect such as brand of product, product appearance, packing size etc. should be produced.

The researcher has realized the great value for human health of phytosterol, which in one of the major components in rice bran oil, contains higher level than other oils such as olive oil, soybean, corn, rape seed and peanut. The benefit of phytosterol could help the patients take less risk of heart disease development, artery blockage and total cholesterol level decrease. The result of studies says that the stability of an oil emulsion in water could cause the higher stable emulsion when using spray dry for encapsulation. This makes the rice bran oil power products contained the amount of total fat higher than 30 percent. Thus, there is the idea of application of rice bran oil powder as several types of food and food ingredients as well.

The data from the survey indicated that tea and coffee products are the choices for the customers who prefer to drink with artificial cream obtained mainly from palm oil products in order to make them tastier (Figure 37). As all details mentioned above, the application of rice bran oil powder in the form of artificial cream has been used with coffee. In order to have a specific idea of the product, the researcher has studied the sensory characteristic and the consumer acceptability for the next study.

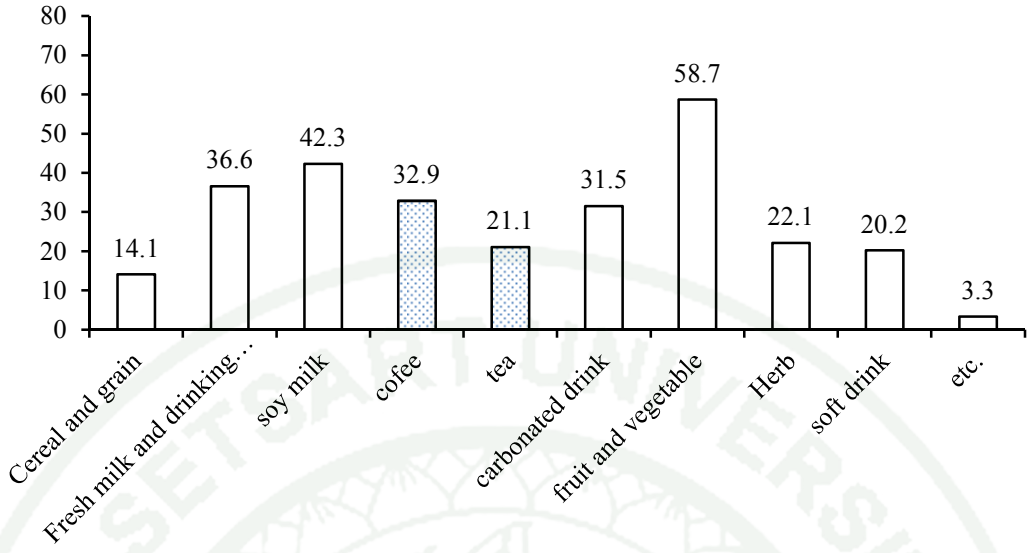


Figure 37 The popular beverage products.

4.3 Descriptive analysis and consumer preference

The 9 trained results of the sensory descriptive analysis (DA) of the non–dairy creamer from powdered rice bran oil stabilized by whey protein (an example 1), modified starch (an example 2), and the commercial non–dairy creamer (4 samples) were obtained. (The sensory descriptor definitions and references for each characteristic were showed in Appendix E). The results showed that there were fifteen attributes of commercial and rice bran oil powder samples. As shown in Table 28, these fifteen attributes can be divided into 2 groups, powder group (5 attributes) and creamer solution group (10 attributes).

Table 28 The characterization of dry powder and creamer solution.

Powder characteristics	Solution characteristics
Color	Color
Hand feel	Solubility
Caking	Opaque
Rice bran oil odor	Oil surface
Milk odor	Rice bran oil odor
	Milk flavor
	Dry grass flavor
	Rice cooked flavor
	Rice bran oil flavor
	Mouth coating

Table 29 Means of sensory attributes of coffee creamer.

Attributes	Samples (commercials and rice bran oil powder samples)					
	1	2	3	4	5	6
Color	1.44±0.73^{bc}	1.88±0.58^{b}	2.29±0.76^{b}	4.50±0.78^{a}	0.78±0.36^{c}	2.27±0.44^{b}
Hand feel	1.22±0.44^{c}	2.00±0.89^{c}	10.07±1.86^{b}	11.63±1.82^{a}	2.00±0.67^{c}	2.22±0.56^{c}
Caking	1.83±0.43^{c}	2.00±0.80^{c}	10.07±1.02^{b}	11.06±1.01^{a}	1.39±0.11^{c}	1.89±0.78^{c}
Rice bran oil odor	0.22±0.05^{c}	0.25±0.03^{c}	4.36±0.68^{b}	5.88±1.07^{a}	0.06±0.01^{c}	0.11±0.03^{c}
Milk aroma	6.22±1.17^{a}	6.69±1.62^{a}	1.71±0.35^{b}	1.19±0.09^{b}	5.72±1.31^{a}	6.17±1.62^{a}
Color	10.00±0.94^{a}	10.00±1.58^{a}	2.64±0.78^{b}	2.69±0.84^{b}	10.28±1.65^{a}	10.44±1.23^{a}
Wettability	9.00±1.22^{b}	9.19±1.51^{b}	9.93±1.43^{b}	11.75±1.03^{a}	11.61±1.02^{a}	12.94±1.29^{a}
Solubility	13.00±1.27^{a}	12.44±1.43^{a}	5.79±1.87^{c}	8.19±1.88^{b}	13.33±0.75^{a}	12.89±0.96^{a}
Opaque	8.94±1.4^{2a}	8.69±0.96^{a}	3.57±0.84^{c}	5.56±1.15^{b}	8.33±1.49^{a}	9.17±1.27^{a}
Oil surface	0.00^{c}	0.06±0.01^{c}	2.21±0.40^{b}	3.44±1.10^{a}	0.17±0.05^{c}	0.61±0.36^{c}
Sweet	0.56±0.46^{ns}	0.75±0.53^{ns}	1.00±0.96^{ns}	0.38±0.35^{ns}	0.56±0.39^{ns}	0.61±0.33^{ns}
Rice bran oil odor	0.06±0.02b	0.06±0.02b	3.43±1.02a	2.94±0.94a	0.11±0.03b	0.11±0.03b
Milk flavor	3.28±1.25a	3.25±1.02a	1.07±0.77b	0.50±0.27b	3.00±1.06a	3.72±0.97a
Dry grass flavor	0.06±0.02b	0.06±0.02b	1.64±0.63a	1.81±0.75a	0.06±0.02b	0.06±0.02b
Rice cooked flavor	0.11±0.02b	0.13±0.03b	3.21±0.81a	3.25±1.21a	0.11±0.03b	0.06±0.01b
Rice bran oil flavor	0.28±0.04c	0.25±0.04c	2.86±0.38b	3.63±0.79a	0.56±0.11c	0.33±0.11c
Mouth coating	1.22±0.51c	1.56±0.78bc	2.36±0.69b	3.44±1.09a	2.00±0.83bc	1.67±0.75bc

[a] = Means in rows followed by different letters represent significant difference ($P \leq 0.05$).

4.3.1 Principal component analysis (PCA)

Principal component analysis is the method of statistic that has chosen or taken the same structure out from the matrix of variance and the variance – covariance matrix in order to create the linear relationship of original data by explaining the highest of variance with the least loss of data. The analyzed data was showed in the form of the relationship table of new component created from the original variance, or showed in the form of PCA chart.

The data of descriptive analysis and physical properties were analyzed by PCA technique. The result of analysis with PCA technique showed that the data of sensory attributes could be used to explain 96.06 % of the total amount of variance. PCA diagram of three examples (CM, WPI and MS) was showed in Figure 38. PC1 described 87.29 % of variance, where yellowness (YE), hand feel (HF), caking (Ca), rice bran oil odor (RBOP), oil surface (OS), rice bran oil odor (RBOR), dry grass flavor (RCO), rice cooked flavor (RCF), rice bran oil flavor (RBOF) and mouth coating (Mtc) positively related with the PC1 axis, while milk aroma (MO), white color (WH), solubility (SO), opaque (OPQ) and milk flavor (MF) negatively related with PC1 axis. For PC2 describing 8.61% of variance, wettability related with PC2 in positive axis.

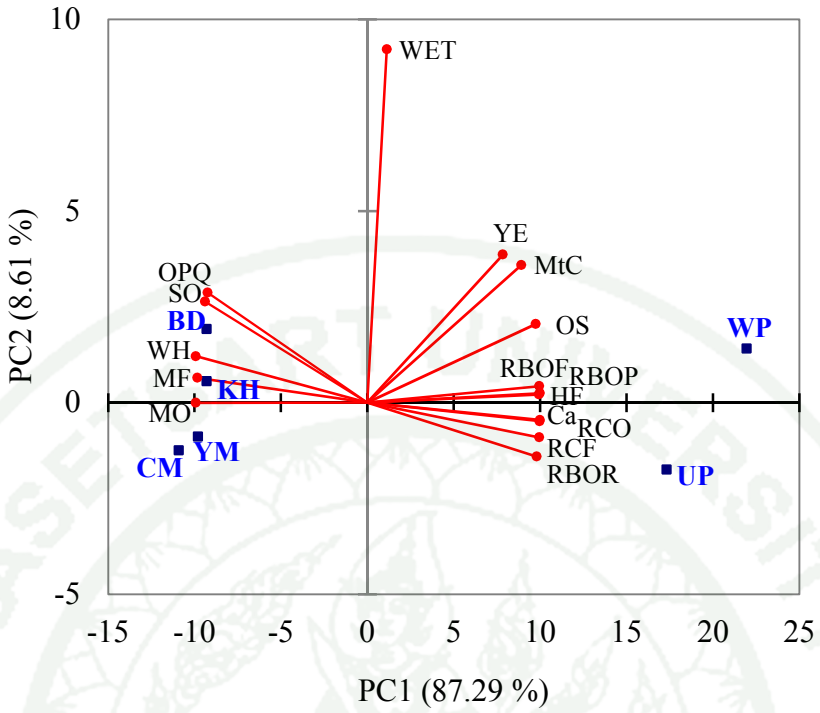


Figure 38 A biplot of the principal component (PC) between all descriptive sensory attribute and the product.

The result of principal component analysis on physical properties data showed that variance could be explained 75.26% of the total amount of variance. The PCA diagram was showed in Figure 39. PC1 described 59.22 % of variance, where b*value (b*), *CI* index (*CI*), *HR* ratio (*HR*) and wettability (WETT) positively related with the PC1 axis, while moisture content (MC), a_w (aw), a* value (a*), bulk density (Bden) and temperature (Tden) negatively related with PC1 axis. For PC2 which described 16.04 % of L* value and total fat content (TTF) variance negatively related with PC2 axis.

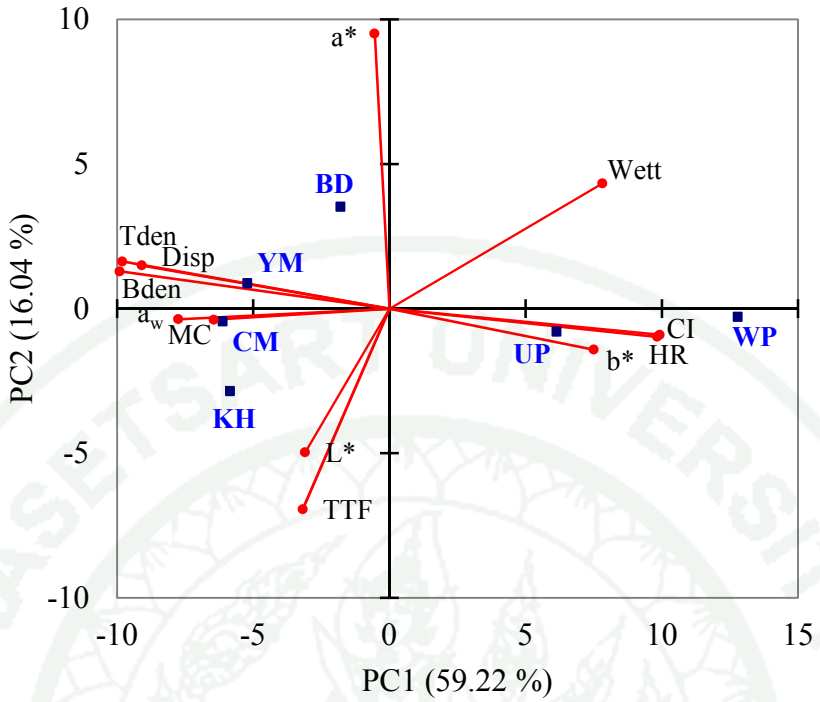


Figure 39 A biplot of the principal component (PC) between physical properties and the product.

The results of descriptive sensory analysis by highly trained panelists showed that there were fifteen attributes contributed to color (powder and coffee creamer), caking, hand feel, solubility, opaque, oil surface, rice bran oil odor (powder and coffee creamer), milk flavor, dry grass flavor, rice cooked flavor, rice bran oil flavor, milk flavor and mouth coating. All fifteen sensory attributes were significantly different from those of coffee creamer samples ($P \leq 0.05$). The differences and relationship between sensory data and coffee creamer samples were described by the first two PCs. The PC1 related to degree of rice bran oil, while the PC2 related to texture properties (wet) for the sensorial parameter. However, in physical parameter The PC1 related to physicochemical properties while the PC2 related to lightness and total fat content. This information could be then applied to consumer research, product development and quality control of end products, in order to provide the direction for product concept of the coffee creamer production.

4.3.2 Consumer preference and just about right

Using the central location test (CLT) and 122 consumers, the results of the consumer preference of rice bran oil powder were obtained. Two different rice bran oil powder samples and the commercial (WPI, MS and CM) were tested using hedonic 9 point scale. Table 30 showed the mean score observed for each product attributes including color, caking, rice bran oil odor (RBO_Odor), milk flavor (Milk_FL), rice bran oil flavor after test (RBO_AF), mouth coating and overall liking. Moreover, using Binomial test, JAR for each product attributes were obtained and shown in Table 31. Binomial test was applied under the hypothesis whether that the total score of too weak used scale was different from the total score of too strong used scale.

Table 30 Consumer mean score of linking scale (N=122).

Attributes	Coffee creamer		
	Powder–WPI	Powder–MS	Commercial brand
Powder form :			
- White color	5.0±1.6 [c]	6.3±1.5 [b]	7.3±1.1 [a]
- Caking	4.9±1.8 [b]	4.3±1.8 [c]	7.1±1.5 [a]
- RBO_odor	5.4±1.3 [b]	5.3±1.3 [b]	6.0±1.4 [a]
- Milk_FL	5.6±1.4 [b]	5.7±1.4 [b]	6.2±1.4 [a]
In coffee :			
- RBO_AF	4.5±1.8 [b]	4.2±1.7 [b]	6.6±1.3 [a]
- Mouth coating	4.9±1.7 [b]	4.6±1.8 [c]	7.0±1.3 [a]
Overall	5.0±1.6 [b]	4.2±1.8 [c]	7.4±1.6 [a]

The mean scores of color, caking and mouth coating, these mean scores of all three examples were significantly different ($P \leq 0.05$). The mean scores of RBO_odor of all two samples (WPI, MS) were not different to those of Milk_FL and RBO_AF.

In the comparison between the overall liking mean scores all three samples, this mean score of CM was highest (7.4), while that of MS was lowest (4.2).

4.3.2.1 Just about right

To investigate which product attributes should be improved, the JAR for the attributes of rice bran oil powder products were explored in term of percentage. The product attributes with more than 70% of JAR means that they were accepted by consumers and were not needed to be improved. For the product attributes with less than 70% of JAR, they were analyzed further using binomial test in order to improve the products.

From Table 31, the results showed that in commercial product, the JAR percentage of color, caking, RBO_AF and mouth coating were more than 70% so they were not needed to be improved. For RBO_odor and Milk_FL, their JAR percentage were less than 70% so they were applied for binomial test.

According to the results of binomial test, the mean scores of caking, RBO_odor, Milk_FL, RBO_AF and Mouth coating of WPI and MS were not JAR. Indeed, the Milk_FL of both WPI and MS were not enough, thus these values should be improved to be higher. On the other hand, the mean score of caking, RBO_odor, RBO_AF and mouth coating of WPI and MS were found to be too much so these values should be improved to be lower. Moreover, since the score of JAR of mouth coating for both WPI and MS were not clear, their meaning could not be concluded, Hence, the consumer test should be repeated or the number of consumer should be increased.

Table 31 JAR consideration.

Sample	Attributes	JAR (%)	Is JAR ≥70	Too weak (n; A)	Too much (n; B)	Sum	Max	Critical value	Are A different the B at $P = 0.05$
WPI	Color	29.51	No	30	56	86	56	53	Yes
	Caking	27.87	No	5	83	88	83	54	Yes
	RBO_odor	47.54	No	16	48	64	48	41	Yes
	Milk_FL	35.25	No	74	5	79	74	49	Yes
	RBO_AF	27.87	No	12	76	88	76	54	Yes
	Mouth coating	46.72	No	26	39	65	39	41	N/A
MS	Color	81.97	Yes						
	Caking	24.59	No	6	86	92	86	56	Yes
	RBO_odor	48.36	No	31	32	63	32	40	No
	Milk_FL	45.9	No	61	5	66	61	42	Yes
	RBO_AF	26.23	No	14	76	90	76	55	Yes
	Mouth coating	45.08	No	28	39	67	39	42	N/A

Table 31 (Continued)

Sample	Attributes	JAR (%)	Is JAR ≥70	Too weak (n; A)	Too much (n; B)	Sum	Max	Critical value	Are A different the B at *P = 0.05*
Commercial product	Color	88.52	Yes						
	Caking	88.70	Yes						
	RBO_odor	65.57	No	41	1	42	41	28	Yes
	Milk_FL	57.38	No	51	1	52	51	34	Yes
	RBO_AF	84.43	Yes						
	Mouth coating	91.8	Yes						

4.3.2.2 Consumer preference

According to the consumer acceptability results shown as followed: The acceptability of the commercial product has the highest percentage of 97.54, followed by WPI (52.46%) and MS (39.34%), respectively.

In addition, the use of WPI and MS as the stabilizer was found to result in the color of rice bran oil powder and the odor of crude rice bran oil, which could not be avoided during the extraction. For caking, it can be improved with the agglomeration after drying method. Moreover, it can be noticed that the use of crude rice bran oil resulted in rice bran oil odor, rice bran oil flavor after taste of the products, and it also resulted in the higher level of the color and caking of all the products, compared with those of commercial products.

The results from sensory attribute analysis with GDA technique suggested that the odor and favor of rice bran oil product can be divided into many groups when it was compared with commercial creamer. Moreover, when either WPI or MS was used as emulsifier, the favor of the product sample was not familiar by consumer. According to the JAR results, consumer suggested that the color favor and odor of the product sample should be improved.

CONCLUSION AND RECOMMENDATION

Conclusion

The rice bran oil qualities including bioactive compounds (e.g., tocopherols, tocotrienol and total phytosterols) were affected by extraction method. Cold pressed extraction gave higher bioactive compounds than that of solvent extraction, while the rice bran types (Khao–dawk–mali 105 and mixed–bran) had slightly effect on those bioactive compounds. Hence, Khao–dawk–mali 105 oil extracted by cold pressed method was selected for the study of the encapsulated rice bran oil.

Biopolymer types had an effect on the formation and stability of rice bran oil in water emulsion. When considered the emulsifier concentrations, at relatively low emulsifier concentrations (emulsifier–to–oil ratio < 0.1), WPI was able to produce the smallest droplets during homogenization, then MS, and then GA. On the other hand, at relatively high emulsifier concentrations (emulsifier–to–oil ratio > 1), MS was able to produce the smallest droplets, then WPI, and then GA. Emulsions formed using the polysaccharide emulsifiers (MS and GA) had much better stability to environmental stresses (pH, salt and thermal processing) than those formed using the globular protein emulsifier (WPI). For the oxidative stability of rice bran oil–in–water emulsions study, the results found that the stability depends on pH and the nature of the biopolymer used to stabilize them. Rice bran oil emulsions containing MS– and WPI–stabilized lipid droplets were relatively stable to lipid oxidation, whereas those containing GA–stabilized droplets were highly unstable to oxidation. The addition of a pro–oxidant (iron/EDTA complex) greatly accelerated lipid oxidation in the GA–stabilized emulsions at both pH 3 and 7, but only accelerated oxidation in the MS– and WPI–stabilized emulsions at neutral pH. The results are interpreted in terms of the impact of biopolymer–iron interactions on the ability of cationic iron ions to interact with emulsified lipids.

The suitable concentrations of WPI or MS (3.5% and 7.0%, respectively) could be used to form emulsions containing small oil droplets (d_{43} < 300 nm) by homogenization of 10% crude rice bran oil. The powder formed by spray drying contained relatively small particles (d_{43} < 25 μm). However, handling and reconstitution properties of the powder, i.e. flowability and wettability were poor to fair probably due to the presence of fine particles within the powder. The result of sorption characteristics of powder stabilized by WPI or MS showed that GAB model was suitable for describing the relationship between the equilibrium moisture content and water activity. The kinetics of lipid degradation of rice bran oil in powder stabilized by WPI or MS was determined as a function of temperature. WPI gave higher encapsulation efficiency (%EE) with slower lipid oxidation rate than MS.

The conclusion from the consumer's survey behavior of having products contained rice bran oil revealed that consumers were not familiar with the new products which were not variously available in the market. As a matter of fact, new products of rice bran oil with suitable product properties aspect such as taste, nutrition value etc. and marketing aspect such as brand of product, packing size etc. should be produced. The results of the used of rice bran oil powder as a coffee creamer showed that the sensory properties of rice bran oil powder including color, odor and favor were needed to be improved to be similar to those of commercial coffee creamer. This can be done by adding whitening agent, emulsifiers, stabilizing salt, favor and color agents etc.

Recommendation

1. The quality control of rice bran is necessary because the amount of bioactive substance is varied according to the storage time of rice bran. Therefore, the pretreatment of rice bran and raw material should be used. For example, the method used to inactivate enzyme before storage, the selection of the package and the low temperatures storage are needed to be considered. All of these factors also affect the quality of rice bran oil and the amount of active ingredients in the oil.

2. Since the sensory characteristics of the rice bran oil powder can vary with the type of biopolymer, there should be studied more about the use of other types of biopolymers on the stability of rice bran oil in water emulsions. The study here showed that whey protein isolate and modified starch were used as the biopolymer, the consumers were not familiar with the appearance of the rice bran oil such as color and flavors of products.

3. The particles of rice bran oil powder encapsulated with a spray dry technique were very small (<25 μm) so these small particle affected the solubility and cohesiveness of the products. Therefore, in the commercial production, the agglomeration technique is necessary to improve the powder properties in order to make the dry powder easier to use. For the further research, the effect of binding agent and the optimization of agglomeration condition should be studied.

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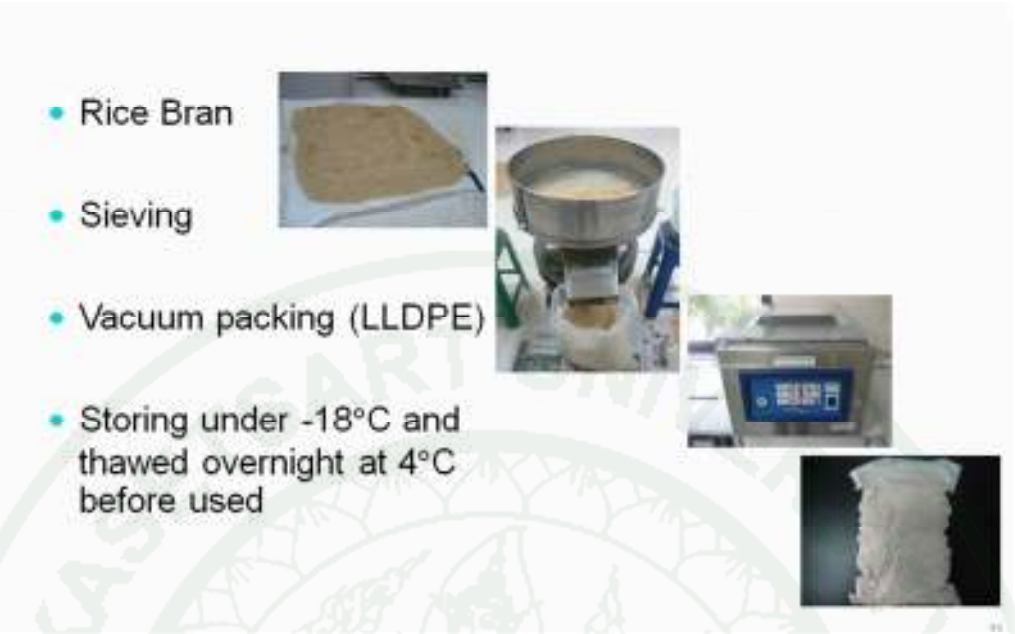
APPENDICES

Appendix A

The preparation of rice bran

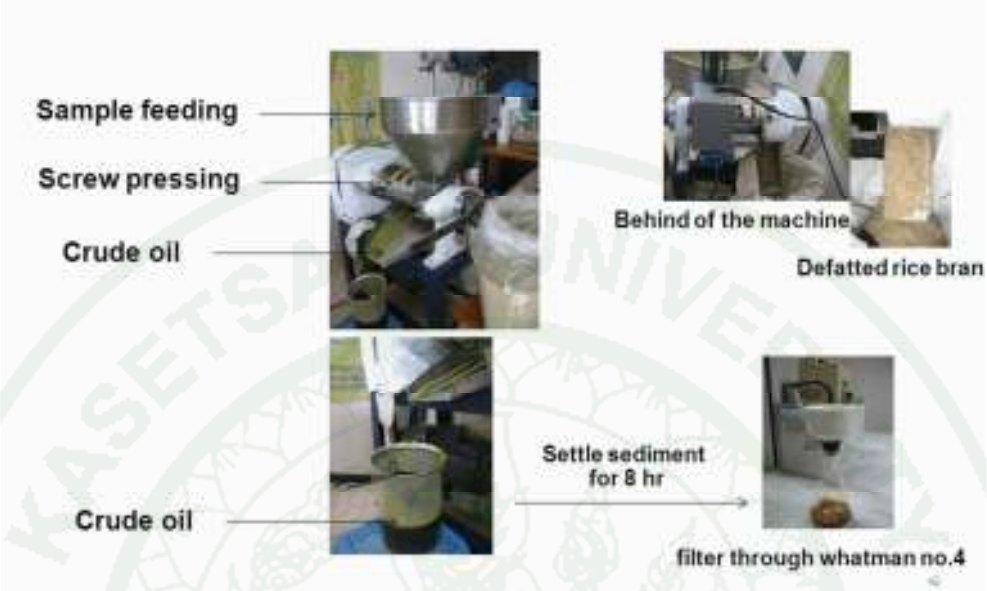


Appendix Figure A1 The preparation of rice bran before used.

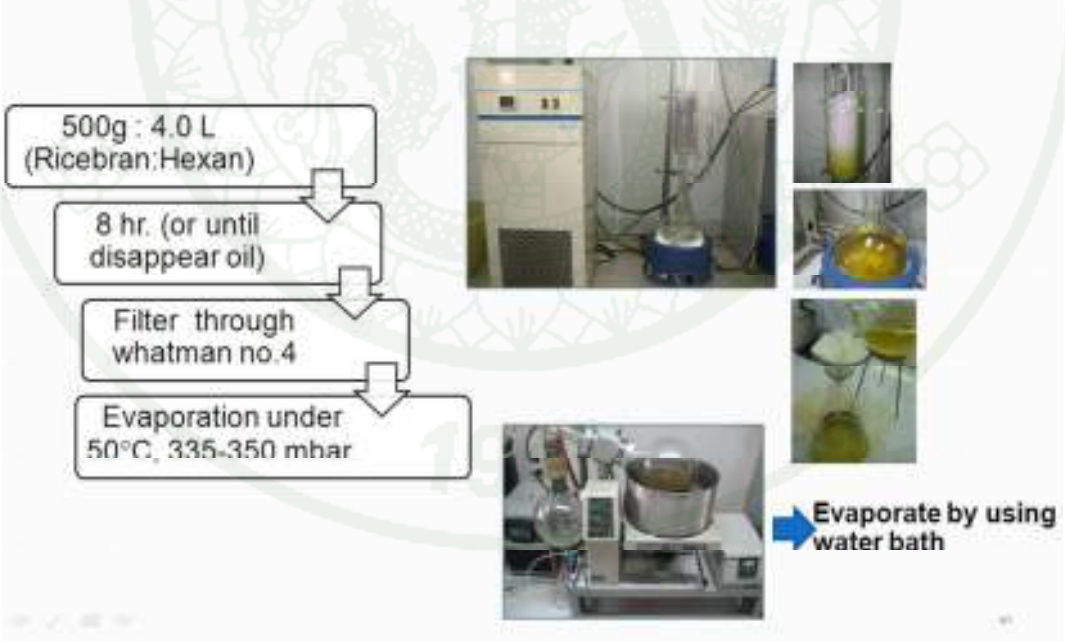
Appendix B

Oil extraction and the quality measurement

a) Cold pressed extraction



b) Solvent extraction



Appendix Figure B1 Oil extraction methods (a) cold pressed extraction and (b) solvent extraction.

Appendix Table B1 Oil extraction conditions.

Process	Solvent extraction	Cold pressed extraction
Raw material	Bran 500 g : hexane 400 mL	Top feeding
Extraction apparatus	Semi continuous extract (soxhlet extraction) by using hexane	Screw press extraction
Extraction condition	Extract oil under heat temperature (60 °C) around 8 h	High pressure under screw (cylinder type), residual bran temperature out 62–67 °C
Oil after extracted	Remove hexane out under rotary evaporator 200–335 mbar, at 60–75 °C	Filter by passed thought filter paper no.1 and no.91, kept in brown bottle and stored under freezing temperature
Sediment separation	Centrifuge at 7000 rpm 15 min. by using refrigerator centrifuge	Centrifuge at 7000 rpm 15 min. by using refrigerator centrifuge
Oil storage	Keep in brown bottle and stored under freeze temperature	Keep in brown bottle and stored under freezing temperature

(a) Oil color measurement

Standard method	:	**AOCS Cc 13e-92**
Glass Cell	:	1 inch
Procedure	:	-
Equipment	:	Lovibond PFX990
Condition	:	Illuminant C and 2°observation
Calculations	:	Y+5R

(b) Refractive index

Standard method	:	**AOCS**
Sample	:	1 droplet
Procedure	:	-
Equipment	:	Refractometer (Abbe'), ATAGO
Condition	:	40 degree celsius

Appendix Figure B2 Oil quality measurement (a) color and (b) refractive index.

(a) Saponification

-sample 4-5 g. + alcoholic KOH 50 mL

-connect the air condenser and boil until completely saponified.

-add phenolphthalein and titrate with 0.5N. HCl until pink color disappears.

-Calculations:

Saponification value

$$= \frac{(\text{vol.of HCl blank} - \text{Vol.of HCl sample}) \times N \times 56.1}{\text{Mass of sample (g.)}}$$

(b) Unsaponification

-5 ±0.1 g sample+ 30 mL 95%Ethanol + 5 mL KOH50%

-Reflux for 1 hr until completely saponified

-Transfer to separatory funnel, Repeat the extraction at least 6 times using 50 mL of petroleum ether each time and shaking vigorously

-Wash the combined extracts 3 times using 25 mL of 10%ethyl alcohol and wash until solution not gives a pink color after drop of phenolpthalein

-Drying to constant weight in vacuum oven at 78C 2.66 mbar. (A)

-Titrate with 0.02N NaOH on the residue in 50 mL with warm 95%alcohol (B)

-The blank determined by same procedure without any fat or oil (C)

-Calculations :

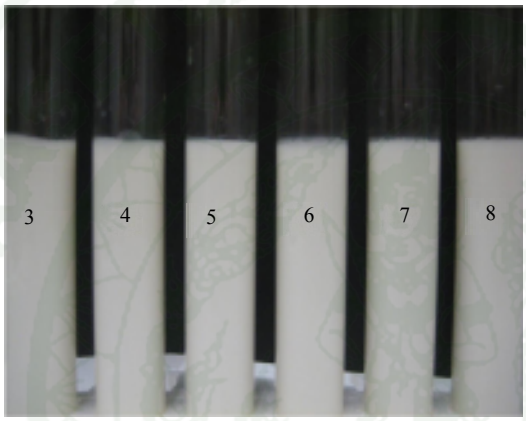
$$\text{Unsaponifiable matter, \%} = \frac{A-(B+C)}{\text{mass of sample, g}} \times 100$$

Appendix Figure B3 Oil quality measurement (a) saponification procedure and (b) unsaponification procedure.

Appendix C

Emulsion stability

(a) whey protein isolate



(b) gum arabic

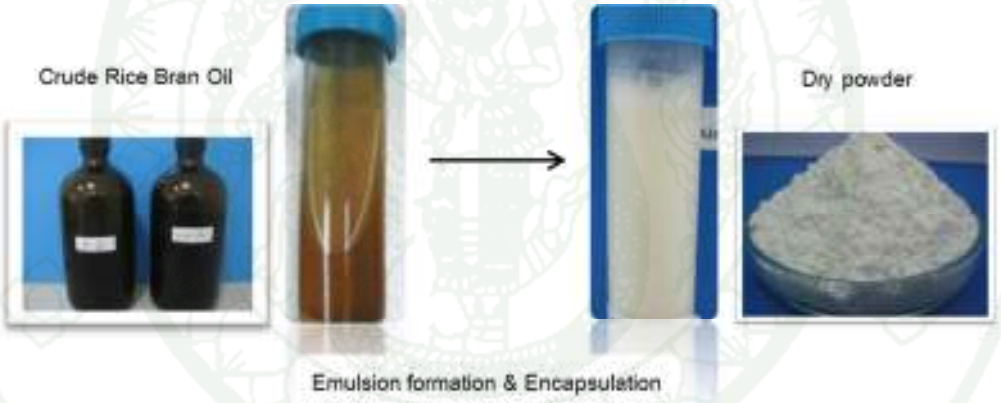
(c) modified starch

Appendix Figure C1 Emulsion stability stabilized different biopolymer, (a) whey protein isolate (WPI), (b) gum arabic (GA) and (c) modified starch (MS).

Appendix D

Rice bran oil and encapsulation

Appendix Figure D1 Oil extracted by different method, (left) cold pressed and (right) solvent.



Appendix Figure D2 Rice bran oil encapsulation.

Pre–homogenization

High pressure homogenizer at 5,500 psi

Emulsion solution

Spray dryer was operated at 180 °C for air inlet
and 90 °C for air outlet

Appendix Figure D3 The encapsulation of rice bran oil by spray dry method.

(a) Rice bran oil stabilized by WPI

(b) Rice bran oil stabilized by MS

Appendix Figure D4 Rice bran oil powder stabilized by different biopolymer.

Appendix E

The questionnaire and sensory evaluation

แบบทดสอบความชอบผลิตภัณฑ์ครีมเทียม

รหัสตัวอย่าง.....

ชื่อผู้ทดสอบ..... วันที่.....สิงหาคม 2554

ส่วนที่ 1 ข้อมูลทั่วไป

1. เพศ ☐ ชาย ☐ หญิง

2. อายุ

☐ ต่ำกว่า 21 ปี ☐ 21-30 ปี ☐ 31-40 ปี ☐ 41-50 ปี ☐ มากกว่า 50 ปีขึ้นไป

ส่วนที่ 2 ข้อมูลเกี่ยวกับการยอมรับผลิตภัณฑ์ครีมเทียมจากน้ำมันรำข้าว

1. **คำอธิบาย** กรุณาทดสอบตัวอย่างที่นำเสนอ และให้**คะแนนความชอบ** ในแต่ละคุณลักษณะที่กำหนด โดยขีดเครื่องหมาย ✓ ลงในช่องระดับความชอบ และให้**ระดับความรู้สึก**ตามที่ท่านรู้สึก โดยขีดเครื่องหมาย / ลงในช่องระดับความรู้สึก

ปัจจัย	ระดับความชอบ									ระดับความรู้สึก		
	ไม่ชอบมากที่สุด 1	ไม่ชอบมาก 2	ไม่ชอบปานกลาง 3	ไม่ชอบเล็กน้อย 4	กลาง 5	ชอบเล็กน้อย 6	ชอบปานกลาง 7	ชอบมาก 8	ชอบมากที่สุด 9	น้อยกว่าปกติ 1	ปกติ 2	มากกว่าปกติ 3
ผงครีมเทียม										-	-	-
1) สี												
2) การจับตัวเป็นก้อน												
3) กลิ่นน้ำมันรำข้าว												
4) กลิ่นนม												
ผลิตภัณฑ์ครีมในกาแฟ										-	-	-
1) กลิ่นน้ำมันรำข้าว												
2) ความมันเคลือบลิ้น												
3) ความชอบโดยรวม												

2. ท่านยอมรับผลิตภัณฑ์ครีมเทียมนี้หรือไม่

( ) ยอมรับ ( ) ไม่ยอมรับ

3. ถ้ามีผลิตภัณฑ์ครีมเทียมจากน้ำมันรำข้าวนี้จำหน่าย ท่านจะซื้อหรือไม่

( ) ซื้อ ( ) ไม่ซื้อ

Appendix Figure E1 Consumer acceptability questionnaire.

Appendix Table E1 Definition and reference for coffee creamer products.

ชื่อผู้ทดสอบ .....

วันที่ .....

Attributes/powder	Definition	Reference with their intensities
<u>1) ลักษณะปรากฏ</u>		
1.1 สีเหลือง	ลักษณะสีเหลืองที่ปรากฏของตัวอย่างครีม ประเมินโดยการเทียบกับแผ่นเทียบสีมาตรฐาน	แผ่นเทียบสีมาตรฐาน 9/2, 9/4
1.2 การจับตัวเป็นก้อน	ลักษณะปรากฏของผง เมื่อเทียบกับนมผง	นมผง S–26 จำนวน 5 กรัม
<u>2) การดม</u>		50% rice bran oil (king 5000 ppm.
2.1 กลิ่นน้ำมันรำข้าว	การรับรู้ถึงกลิ่นหรือคล้ายกลิ่นน้ำมันรำข้าว	oryzanol) in 1% WPI
<u>3) การสัมผัสด้วยมือ</u>		
3.1 ความละเอียด	ความรู้สึกสัมผัสต่อผงภายหลังสัมผัสด้วยมือ ระหว่างนมผงและแป้งอเนกประสงค์	นมผง S–26 จำนวน 5 กรัม แป้งอเนกประสงค์ 5 กรัม

Appendix Table E1 (Continued)

Attributes/solutions	Definition	Reference with their intensities
<u>1)ลักษณะปรากฏ</u>		
1.1 สีขาว	ลักษณะสีขาวที่ปรากฏของตัวอย่างครีมที่ละลายในน้ำร้อน ประเมินโดยการเทียบกับแผ่นเทียบสีมาตรฐาน	แผ่นเทียบสีมาตรฐาน ระดับ 9/ , 9.5
1.2 ความทึบแสง	ลักษณะปรากฏที่สัมพันธ์กับระดับของความทึบแสงของ 0.5%,100% whole milk	0.5% whole milk น้ำอุณหภูมิห้อง
1.3 Wettability	สังเกตตัวอย่างผงเมื่อเทลงในบิกเกอร์กำหนดปริมาตร 200 mL : ตัวอย่าง 2.0g.	1.0% rice bran oil ในน้ำกลั่น
1.4 Solubility	สังเกตตัวอย่างภายหลังจากคนตัวอย่างตามเข็มนาฬิกา 10 ครั้ง	
1.5 ปริมาณน้ำมันที่ผิวหน้า	เปรียบเทียบปริมาณน้ำมันที่เกิดขึ้นที่ขอบภาชนะ	
<u>2) กลิ่น</u>		ละลายนมข้นหวาน ½ ช้อนชา ในน้ำอุ่น
2.1 กลิ่นนม	การรับรู้ถึงกลิ่นนม กลิ่นน้ำมันรำข้าวหรือคล้ายคลึงกัน รวมถึงกลิ่นน้ำซาวข้าว	200 มล. และอุ่นตัวอย่างให้อุ่นอยู่เสมอ
2.2 กลิ่นน้ำมันรำข้าว	ภายหลังจากการสูดดมตัวอย่างทันทีเมื่อเปิดฝา	~80 ºC
2.3 กลิ่นน้ำซาวข้าว		ข้าวสาร 1 ส่วน ต่อน้ำ 1 ส่วน เอาน้ำซาวข้าวครั้งที่ 2 และ เติมน้ำลงไปเท่ากับปริมาณน้ำซาวข้าวที่ได้

Appendix Table E1 (Continued)

Attributes/solutions	Definition	Reference with their intensities
<u>3) รสพื้นฐาน</u>		
3.1 รสหวาน	กลิ่นรสพื้นฐาน	สารละลายน้ำตาลซูโครส 2%
<u>4) กลิ่นรส</u>		
4.1 กลิ่นรสนม		นมข้นหวานเจือจาง เหมือน 2.1
4.2 กลิ่นรสน้ำมันรำข้าว		น้ำมันรำข้าวบริสุทธิ์
4.3 กลิ่นรสน้ำซาวข้าว		น้ำซาวข้าวเจือจาง เหมือนข้อ 2.3
<u>5 กลิ่นรสตกค้าง</u>		
- ความมันเคลือบลิ้น	ความรู้สึกถึงความมัน หรือลักษณะมันเคลือบลิ้น ซึ่งเป็นคุณลักษณะเป็นเมือกลื่นหุ้มที่ผิวของลิ้น	0.5% whole milk

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