Chapter 4

Results and Discussion

1. Effect of stabilization of rice bran by domestic heating on mechanical extraction yield, quality, and antioxidant properties of cold-pressed rice bran oil.

To stabilize rice bran by domestic heating for cold-pressed RBO process, experiments were carried out by using hot air, roasting, steaming and microwave heating. The effects of domestic heating on moisture content of rice bran and extraction yield of cold-pressed rice bran oil are shown in Table 4.1 Fresh rice bran (unstabilized) had moisture of 14.56 g/100g bran. The data show that domestic heating could reduce the moisture content of rice bran. The moisture content of steaming, hot air, microwave and roasting of stabilized rice bran were 11.41, 4.58, 4.05 and 2.12 g/100g bran, respectively. Oil extraction yield from rice bran increased according to the application of hot air, roasting and microwave heating ($P < 0.05$) (Table 4.1). The oil extraction yield of stabilized RBO by hot air, microwave, roasting and steaming were 5.53, 4.81, 4.77 and 3.41 g/100g bran, respectively. The stabilization of rice bran by steaming had no significant difference on oil extraction yield from the ustabilized rice bran ($P < 0.05$). This suggests there exists an optimal moisture content for domestic heating and the provision of a higher moisture content significantly impact on the extraction yield. The low moisture in the stabilized rice bran can make them more brittle and therefore can achieve a greater rupture of tissue and increase the extraction of oil during the mechanical pressing (Uquiche *et al*., 2008). There are two types of lipids in oilseeds: storage lipids, which are mainly triacylglycerols and which are high in quantity and localized in oil bodies of the tissues; and membrane lipids, which are mainly phospholipids (Liu and Brown, 1996). The heating may modify the cellular wall, which results in greater porosity. It also could vaporize the water of the vegetable substrate microstructure, increasing the pressure in its interior; its release causes the disintegration of the material (Starmans and Nijhuis, 1996;

Aguilera and Stanley, 1999), cell membrane rupture and improves the efficiency of the pressing extraction of oil from oilseeds, enabling the passage of oil from the cell membrane (Uquiche *et al*., 2008). Our results were in agreement with other plants oil extraction. Azadmard-Damirchi *et al*., (2010) reported that pretreatment of rapeseed with microwave heating could enhance oil extraction yield of cold pressing and solvent extraction methods. Oil extraction yield from hazelnut seed increased according to the application of microwave heating (Uquiche *et al*., 2008).

Extraction of RBO has been performed using conventional techniques like solvent extraction and mechanical pressing. But solvent extraction (hexane) is more commonly used in the RBO industry. These techniques usually use organic solvents which are expensive and sometimes toxic. Solvent oil extraction is the most efficient method; however, its application presents some industrial disadvantages such as plant security problems, emissions of volatile organic compounds into the atmosphere, high operation costs and poor quality products caused by high processing temperatures (Uquiche *et al*., 2008). In our previous work, we studied on yield and chemical properties of solvent extracted and cold-pressed RBO (*Oryza saltiva* L.) Rice bran sample were stabilized by hot air heating (hot air oven) at 150 °C for 10 min prior to extraction with hexane and pressing. We found that solvent extraction had higher the extraction yield of oil than that of cold pressing method. But cold-pressed RBO had lower values in acid, peroxide and free fatty acid but higher iodine number than that of solvent extracted RBO. Therefore, the cold-pressed extraction process gave a better crude oil quality and contained higher vitamin, phytochemical and mineral content than that of solvent extraction.

Table 4.1 Effect of stabilization of rice bran by domestic heating on moisture content of rice bran and extraction yield of cold-pressed rice bran oil.

Stabilization methods	Moisture (g/100g bran)	Extraction yield (g/100g bran)
Unstabilized	14.56 ± 0.08 [aA]	3.29±0.23[c]
Hot air	4.58±0.51[c]	5.53±0.16[a]
Roasting	2.13±0.04[e]	4.77±0.30[b]
Steaming	11.41±0.04[b]	3.41±0.14[c]
Microwave	4.05±0.13[d]	4.81±0.24[b]

[A] Values (means ± SD) with different index letters are statistically significantly different ($P < 0.05$).

Color is another important characteristic for determining visual acceptance of RBO. The influences of domestic heating on quality of cold-pressed RBO were studied. Color of RBO was determined by CIE system (Table 4.2). The L* value is the "lightness" of a sample from 0 to 100 with 100 being pure white; the a* value describes red (+) to green (–); the b* value represents yellow (+) to blue (–); and zero values for "a*" and "b*" represent gray. The RBO differed in their colors. Significant different were found between L*, a* and b* values of RBO ($P < 0.05$). Roasting and steaming could reduce the lightness of RBO and all of the heating methods could reduce the b* value of RBO ($P < 0.05$). However, there are no color standards for cold pressed rice bran oil and the L*, a* and b* measurement could thus be used for color classification.

Table 4.2 Effect of stabilization of rice bran by domestic heating on color of cold-pressed rice bran oil.

Stabilization methods	Color		
	L*-value	a*-value	b*-value
Unstabilized	10.44±0.27 [aA]	1.59±0.06[cd]	12.94±0.32[a]
Hot air	9.69±0.86[a]	2.84±0.60[b]	10.66±0.37[b]
Roasting	7.99±0.54[b]	3.72±0.50[a]	8.80±1.00[b]
Steaming	6.49±0.52[c]	1.40±0.39[d]	8.90±0.58[b]
Microwave	10.09±0.92[a]	2.28±0.33[bc]	9.65±1.85[b]

[A] Values (means ± SD) with different index letters are statistically significantly different ($P < 0.05$).

Stabilization of bran to inactivate enzyme activity is the most important factor in rice bran oil extraction. Poor or no stabilization causes the increase in AV, PV and FFA content and affects the extraction process, oil quantity and quality. The effects of stabilization of rice bran by domestic heating on chemical properties of cold-pressed RBO are shown in Table 4.3. Acid value (AV), free fatty acid (FFA) and peroxide value (PV) were the parameters used for determination of chemical quality of cold-pressed RBO. Generally, the content of AV, FFA and PV was positively related to the activity of enzyme lipase. Acid value can be used for a purity check of oil and may have already started decomposition reactions. Although refined oils are largely devoid of free fatty acids, considerable amounts may be present in crude oils. Hydroperoxides are the primary products of autoxidation which in themselves are odorless. Their decomposition leads to the formation of a wide range of carbonyl compounds, hydrocarbons, furans and other products that contribute to the stale flavor of foods and may also be involved in biological oxidation

(Frankel, 1991). The peroxide value (PV) measures the quantity of peroxides in the oil; these are important intermediates of oxidative reactions since they decompose via transition metal irradiation and elevated temperatures to form free radicals (Decker, 1998). Data show that different domestic heating methods provided different chemical qualities of cold-pressed RBO ($P < 0.05$). Steaming, roasting, hot air and microwave heating could retard the forming of AV, FFA and PV compared with unstabilized rice bran. Cold- pressed RBO of hot air and microwave heating had lower AV, FFA and PV than that of roasting and steaming methods, respectively. However, there was no significant difference between the rice bran samples treated with hot air and microwave heating ($P < 0.05$). This may be due to the fact that, in this condition, the heat could penetrate and effectively destroy lipase and it combined with the prevention effect of rice bran to retard the development of oxidation products in rice bran. Stabilization of rice bran with hot air and microwave heating are effective methods for controlling enzyme activity in rice bran. These results indicate that the stabilization of rice bran by domestic heating can be employed without concern as to deleterious changes to major nutrient concentrations in the bran. Our result is in agreement with Ramarathnam *et al*.(1989) who reported that fatty acid and proximate compositions did not change drastically in microwave-heated rice bran compared with raw samples kept under similar storage conditions. Amarasinghe *et al*. (2009) studied the effect of method stabilization on aqueous extraction of rice bran oil. They found that steaming, microwave and hot air heating could retard the lipolytic activity of rice bran resulting in the lower FFA when compared with unstabilized rice bran.

According to CODEX standards for edible fat and oil (CODEX STAN 210-1999), the maximum level of AV and PV of cold pressed oil are 4.0 mg KOH/g oil and 15 milliequivalents of active oxygen/kg oil, respectively. According to Tao *et al*. (1993), rice bran oil with over 5% FFA is considered unsuitable for human consumption. Our results show that stabilized RBO by microwave and hot air heating had AV 6.30-6.98 mg KOH/g oil, FFA 3.17-3.51% and PV 11.72-12.13 milliequivalents of active oxygen/kg oil. The stabilized rice bran with hot

air and microwave had PV lower than that of CODEX standards and had FFA 5% lower but they had a little higher AV than that of CODEX standards. Therefore, the stabilized rice bran by hot air and microwave heating could be applied to produce the cold pressed RBO for human consumption. But it may need to find some process to reduce AV to reach the CODEX standard.

Rice grain contains several classes of antioxidants, including phenolic compounds, tocols and gamma oryzanol. Antioxidants reportedly are protective against oxidative damage, which has been implicated in a range of diseases, including cancer and cardiovascular disease. They are also one of the principal ingredients that protect food quality by preventing oxidative deterioration of lipids (Goffman and Bergman, 2004). Cold-pressed edible seed oils may be preferred by consumers because the cold pressing procedure involves neither heat nor chemicals, and may increase the retention of beneficial phytochemicals (Yu *et al*., 2005). It is well accepted that antioxidants may protect important cellular components such as DNA and membrane lipids from oxidative damage and suppress the pathology of cancer, cardiovascular diseases, and other aging-associated health problems. RBO rich in natural antioxidants may play a role in reducing the risk of chronic diseases. Phenolic compounds have demonstrated powerful antioxidative potential and may reduce free radical-mediated cellular damage. Phytochemical contents of stabilized cold-pressed RBO as compared to unstabilized cold-pressed RBO are displayed in Table 4.4. Results showed the total phenolic content ranging from 11.59 to 11.27 mg FAE/g oil, flavonoid content ranging from 9.04-12.18 mg CE/g oil and gamma oryzanol content ranging from 2.03-2.25 g/100g oil. Stabilized rice bran with domestic heating yielded significantly higher amounts of total phenolic compounds, flavonoid content, gamma oryzanol than that of unstabilized rice bran ($P < 0.05$). However, steaming stabilized RBO had no significant difference in flavonoid content when compared with unstabilized RBO ($P > 0.05$). Microwave and hot air heating stabilized RBO contained higher contents of total phenolic compounds than that of roasting and steaming stabilized RBO. Cold-pressed RBO of hot air heating had the highest content of gamma oryzanol but there were not significantly different in microwave, roasting and steaming

($P < 0.05$). Rice bran contains a significant amount of natural phytochemicals such as oryzanols, tocopherols and tocotrienols that have been reported as the strongest antioxidants in rice bran (Lai *et al*., 2009; Godber and Wells, 1994; Orthoefer and Eastman, 2004). The total phenolic compound, flavonoid content and gamma oryzanol content of the rice bran stabilized by domestic heating were comparable to those of rice bran reported by Chotimarkorn *et al.* (2008) and Lai *et al.* (2009). Cold-pressed RBO contained total phenolic compound content 1.44 mg CAE/100 g (Singer *et al*., 2008).

Table 4.3 Effect of stabilization of rice bran by domestic heating on chemical property of cold-pressed rice bran oil.

Stabilization methods	Chemical characteristic		
	Acid value (mg KOH/g oil)	Free fatty acid (%)	Peroxide value (mg Eqv/kg oil)
Unstabilized	11.11±0.84 [Aa]	5.58±0.42[a]	18.85±0.45[a]
Hot air	6.98±0.31[cd]	3.51±0.16[cd]	12.13±0.22[d]
Roasting	7.56±0.03[c]	3.80±0.01[c]	15.18±0.50[c]
Steaming	9.01±0.40[b]	4.53±0.20[b]	17.16±0.59[b]
Microwave	6.30±0.55[d]	3.17±0.27[d]	11.72±0.59[d]

[A] Values (means ± SD) with different index letters are statistically significantly different ($P < 0.05$).

Table 4.4 Effect of stabilization of rice bran by domestic heating on phytochemical content of cold-pressed rice bran oil.

Stabilization methods	Phytochemical content		
	Total Phenolic (mg FAE/g oil)	Flavonoid (mg CE/g oil)	Gamma oryzanol (g/100g oil)
Unstabilized	11.59±0.89 [Ac]	9.04±0.12[b]	2.03±0.05[c]
Hot air	15.70±3.35[a]	11.81±1.20[a]	2.30±0.08[a]
Roasting	13.71±2.53[b]	11.75±0.28[a]	2.24±0.04[ab]
Steaming	13.65±1.79[b]	10.01±0.69[b]	2.16±0.05[b]
Microwave	16.27±1.10[a]	12.18±0.65[a]	2.25±0.02[ab]

[A] Values (means ± SD) with different index letters are statistically significantly different ($P < 0.05$).

The effects of domestic heating on antioxidant activity of cold-pressed RBO are shown in Figure 4.1. Free radical-scavenging and inhibition of lipid peroxidation have been studied to explain how RBO could be used as effective antioxidants. The DPPH free radical method has been used extensively to evaluate reducing substances, based on the reduction of methanolic DPPH solution in the presence of a proton-donating substance, resulting in the formation of diamagnetic molecules. The data showed that the stabilized RBO with hot air, roasting, steaming and microwave had DPPH scavenging effect and inhibition on linoleic acid peroxidation than that of unstabilized rice bran ($P < 0.05$). Hot air and microwave heated RBO had the inhibition on linoleic acid peroxidation higher than that roasted and steamed RBO ($P < 0.05$). The results indicated that the rice bran with stabilization process by domestic heating appeared to increase the oxidative

stability expressed by the higher activity than unstabilized rice bran. The stabilized rice bran can reduce lipid oxidation because it contains a number of antioxidants and this result could also be indicated by the antioxidant results and bioactive compounds of RBO as shown in Table 4.4. The antioxidant activity of compounds is often described by its ability to delay the onset of autoxidation by scavenging reactive oxygen species, or the ability to act as chain breaking antioxidants to inhibit the propagation phase of lipid autoxidation (Yuan *et al*., 2005). Chotimarkorn *et al*. (2008) reported that the methanolic rice bran extracts produced strong results with DPPH free radical-scavenging (EC_{50} 0.38–0.74 mg/ml), reducing power (EC_{50} 0.10–0.53 mg/ml), ferrous ion-chelating activity (EC_{50} 0.11– 0.55 mg/ml) and inhibition of lipid peroxidation (EC_{50} 0.14–0.57 mg/ml). Singer et al. (2008) reported that cold-pressed RBO had DPPH scavenging 23%. Additionally, there are very few studies on the effect of stabilization method of rice bran on the phytochemical content and antioxidant activity of RBO, particularly cold-pressed RBO. However, our results have similar phenomena with stabilization method of other plat oils. Azadmard-Damirchi *et al*. (2010) studied the effect of pretreatment with microwave on oxidative stability and nutraceuticals content of rapeseed oil. Microwave pretreatment of rapeseed can increase oil extraction yield, phytosterols and tocopherols of the oil extracted by pressing method. Oil extracted from untreated rapeseed by press had the lowest oxidative stability (1 h); this was increased to 8 h by pretreatment of rapeseed with microwaves. Higher stability of oils extracted from microwave pretreated rapeseeds may arise from their high antioxidant content. Roasted sesame seed oils are widely used in Asian and African countries. Roasting of sesame seed before pressing could increase the phytochemical which results in resistance to oxidative deterioration, distinctive flavor and extended shelf-life of sesame seed oil (Lee *et al*., 2010). Uquiche *et al*. (2008) reported that stabilization of hazelnut with microwave heating prior to pressing could improve the oil recovery, quality and oxidative stability of cold pressed hazelnut oil.

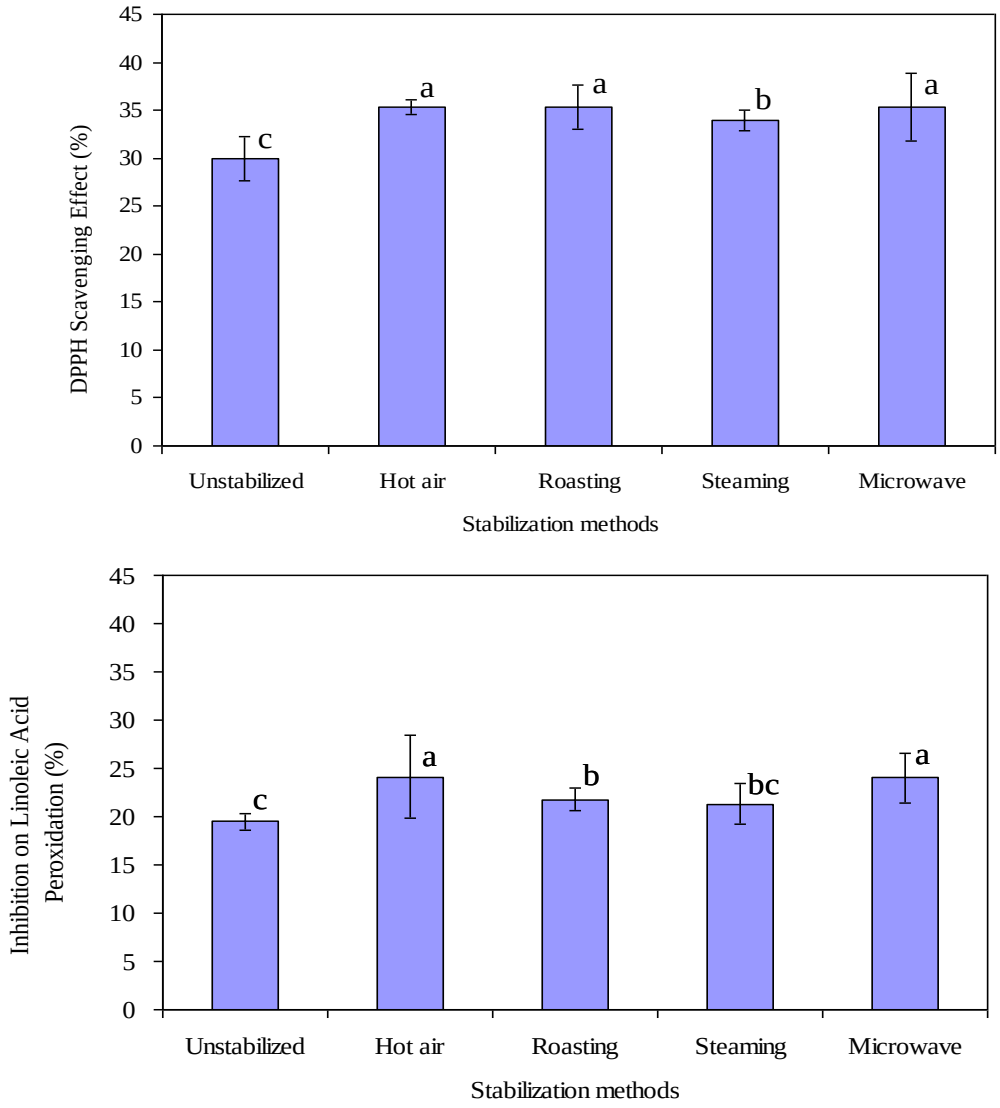

Figure 4.1 Effect of the stabilization of rice bran by domestic heating on DPPH scavenging effect (%) and inhibition on linoleic acid peroxidation (%) of cold-pressed rice bran oil. The different index letters are statistically significantly different ($P < 0.05$).

2. Comparative Study on Yield and Chemical Properties of Solvent Extracted and Cold-Pressed Rice Bran Oil (*Oryza saltiva* L.)

Proximate Composition of Sangyod Rice Bran

The proximate compositions of Sangyod rice bran (SRB) are shown in Table 4.5 SRB contained moisture of 13.40%, crude protein 11.16%, crude lipid 17.31%, crude fiber 9.30%, ash 9.63% and carbohydrate 48.50%. These results are in agreement with other research. Juliano and Hick (1996) reported that rice bran contained 34-62% starch, 11-15% protein, 24-29% dietary fiber and 6.6-9.9% mineral. Rice bran is a satisfactory source of fat with a range between 12-20% (Marshall and Wadsworth, 1994). Rice bran comprised of the aleurone layer of the rice kernel and some part of the endosperm and germ, which are rich sources of proteins, lipids, vitamins, and trace minerals. Rice bran oil may be the largest underutilized agricultural commodity in the world. It is one of the most health-promoting forms of vegetable oil. Rice bran and its oil contain large concentrations of antioxidant compounds such as tocopherol, tocotrienol and gamma oryzanol that could potentially prevent chronic diseases such as coronary heart disease and cancer. It was also reported that RBO has non-allergenic and toxic substances (Hypoallergenic oil). RBO has been used in many foods, neutraceutical and cosmetic applications (Godber, 2008). Therefore, SRB and SRBO could be a promising source of functional ingredients for nutraceutical and functional food applications.

Table 4.5 Proximate compositions of Sangyod rice bran.

Compositions	Content (g/100 g rice bran)
Lipid	17.31 ± 0.10
Moisture	13.40 ± 0.22
Ash	9.63 ± 0.25
Crude fiber	9.30 ± 0.30
Carbohydrate	48.50 ± 0.23
Calorie (kcal)	394.43 ± 0.53

[A] Values are means ± SD from triplicate determinations.

Extraction Yield

Extraction of SRBO is shown in Table 4.6. Hexane was used for the conventional method (solvent extraction) and cold pressing was used as the green process. Data show that the conventional method extraction by hexane with the ratio of SRB to hexane of 1:4, 1:6 and 1:8 gave the oil yield at 4.37%, 7.67% and 7.77%, respectively. Table 2 shows that increasing of SRBO yield with increasing the extraction time up to 45 min (7.53%) ($P < 0.05$). When the extraction time was further to 60 min the oil yield was not increased ($P > 0.05$). From the results, the extraction conditions of SRBO with hexane at the ratio of rice bran to hexane of 1:6 at room temperature for 45 min was selected and used for further experiments. From this experiment, we also found that the cold-press extraction of SRBO had lower yield than that of hexane extraction ($P > 0.05$). Hexane extraction has a low cost, high oil yield but risk of health and safety issues. Conventional methods of rice bran oil extraction are chemical and physical methods. However, the commercial edible rice bran oil is typically extracted with solvents. Hexane is the common solvent used

for extraction of rice bran oil. Although concern for impending regulatory scrutiny of hexane for the environmental and toxicological reasons prompted exploration of other approaches to extraction such as alternative solvents and supercritical fluid extraction. Although extracting of oil from bran is possible through mechanical means via pressing, oil recovery is lower and costs are higher compared to solvent extraction (Godber, 2008). However, the solvent extraction in this research had lower oil yield than in the literature. Zigoneanu *et al*. (2008) reported that the conventional solvent extraction at 40 °C for isopropanol yielded approximately 12% oil of the fresh rice bran, not significantly different from the oil yielded by microwave-assisted extraction under the same conditions. The amount of oil extracted with hexane by conventional solvent extraction was approximately 14% which was similar with the amount of oil extracted with hexane by microwave-assisted extraction (Zigoneanu *et al*., 2008). Proctor and Bowen (1996) reported that a 14.95% oil yield could be extracted from rice bran by conventional solvent extraction using hexane as a solvent at ambient temperature. Hu *et al*. (1996) reported that approximately 19% oil from the fresh rice bran was extracted by hexane at 40 and 60 °C. The differences in oil yield between different data reported in the literature and those found in this study can be explained by a number of factors including rice type, storage, milling, rice bran stabilization process, and extraction conditions (Zigoneanu *et al*., 2008).

Table 4.6 Oil yield of Sangyod rice bran oil.

Method of extraction	Extraction condition	Oil Yield (%)
Cold pressing	-	5.65±0.27 [Ac]
Solvent	Bran : hexane (1:4), 60 min	4.35±0.12[d]
Solvent	Bran : hexane (1:6), 60 min	7.67±0.26[a]
Solvent	Bran : hexane (1:8), 60 min	7.77±0.57[a]
Solvent	Bran : hexane (1:6), 15 min	5.69±0.40[bc]
Solvent	Bran : hexane (1:6), 30 min	6.31±0.36[b]
Solvent	Bran : hexane (1:6), 45 min	7.53±0.30[a]
Solvent	Bran : hexane (1:6), 60 min	7.45±0.08[a]

[A] Values are means ± SD from triplicate determinations.

Values with different superscript letters in the same row indicate significant different ($P < 0.05$).

Chemical Characteristics and Fatty Acid Profile

The results on chemical characteristics of SRBO are shown in Table 4.7. The acid value, peroxide value, iodine number and free fatty acid value were recorded to be 8.32- 10.24 mg Eqv/kg oil, 9.64-12 mg KOH/g oil.19, 95.48-102.28 and 4.18-5.14% as oleic acid, respectively. The data show that the cold-pressed SRBO had lower values in acid, peroxide and free fatty acid but a lower iodine number than that of hexane extracted SRBO ($P < 0.05$). It implied that the cold-pressed SRBO had lower lipid degradation by lipid oxidation and hydrolysis than that of hexane extracted SRBO. Therefore, application of the cold-pressing extraction method for producing of SRBO should be a concern in the small-scale factory. Mezouari *et al*. (2006)

reported that Thai crude rice bran oil had acid, peroxide values and iodine numbers of 12 mg KOH/g oil, 9.80 meq/kg oil and 90, respectively.

The fatty acid profile of the solvent extracted and cold pressed SRBO is shown in Table 4.8. The major fatty acids in SRBO were palmitic acid (21.93-20.10%), oleic acid (41.01-44.20%) and linoleic acid (30.32-30.00%). However, cold-pressed SRBO had higher unsaturated fatty acid than that of solvent extracted SRBO ($P < 0.05$). Lauric acid**,** myristic acid**,** pentadecanoic acid, palmitoleic acid**,** peptadecanoic acid**,** arachidic acid**,** eicosenoic acid, behenic acid and lignoceric acid were present in minor quantities in SRBO. The fatty acid profiles of both SRBO in this research are in agreement with the findings in the literature. Godber (2008) reported that rice bran oil is similar to other vegetable oils. Its fatty acid profile compared with other vegetable oil tends to be higher in oleic acid and lower in linoleic acid. Ramezanzadeh *et al*. (2000) reported that three major fatty acids, palmitic acid (12-18%), oleic acid (40-50%), and linoleic acid (30-35%), make up 90% of the total fatty acids of rice bran oil. Fatty acids are important in a number of functions in the human body. Linoleic acid, with two double bonds, is one of the essential fatty acids and is found at high concentrations in vegetable oil and, to a smaller extent, in meats. Rice bran is a good source of linoleic acid. The amount of linoleic acid in a normal diet is approximately 2% of the total calories. Two to three tablespoons of soybean oil will supply the needed amount. With rice bran oil (30-35% linoleic acid), the required amount is about 3-4 tablespoons per day (Ramezanzadeh et al., 2000).

Table 4.7 Chemical characteristics of Sangyod rice bran oil.

Chemical characteristics of SRBO	Method of extraction	
	Solvent	**Cold Pressing**
Acid value (mg KOH/g oil)	10.24 ± 0.03^{Aa}	8.32 ± 0.01^{b}
Peroxide value (mg Eqv/kg oil)	12.19 ± 0.04^{a}	9.64 ± 0.07^{b}
Iodine number	95.48 ± 0.05^{b}	102.28 ± 0.11^{a}
Free fatty acid (%)	5.14 ± 0.01^{a}	4.18 ± 0.05^{b}

[A] Values are means ± SD from triplicate determinations.

Values with different superscript letters in the same row indicate significant different ($P < 0.05$).

Table 4.8 Fatty acid composition of Sangyod rice bran oil.

Fatty acid of SRBO (g/100 oil)	Method of extraction	
	Solvent	Cold Pressing
Lauric acid (C12:0)	0.05 ± 0.002[A]	ND
Myristic acid (C14:0)	0.37 ± 0.005[a]	0.30 ± 0.003[a]
Pentadecanoic acid (C15:0)	0.03 ± 0.003	ND
Palmitic acid (C16:0)	21.93 ± 0.240[a]	20.10 ± 0.250[b]
Palmitoleic acid (C10:1 n-7)	0.21 ± 0.006[a]	0.10 ± 0.003[a]
Heptadecanoic acid (C17:0)	0.05 ± 0.003	ND
Stearic acid (C18:0)	2.26 ± 0.030[a]	2.00 ± 0.040[a]
Cis-9-Octadecenoic acid (C18:1 n-9) (Oleic acid)	41.02 ± 0.075[b]	44.20 ± 0.067[a]
Cis-9,12-Octadecenoic acid (C18:2 n-6) (Linoleic acid)	30.32 ± 0.095[a]	30.00 ± 0.106[a]
Cis-9,12,15-Octadecenoic acid (C18:3 n-3) (Linolenic acid)	1.56 ± 0.080[a]	1.30 ± 0.030[a]
Arachidic acid (C20:0)	0.84 ± 0.050[a]	0.40 ± 0.003[b]
Cis-11-Eicosenoic acid (C20:1 n-9)	0.52 ± 0.080[a]	0.20 ± 0.008[b]
Behenic acid (C22:0)	0.27 ± 0.026[a]	0.20 ± 0.003[a]
Lignoceric acid (C24:0)	0.39 ± 0.003[a]	0.40 ± 0.005[a]
Total monounsaturated fatty acid	41.75	44.50
Total polyunsaturated fatty acid	31.88	31.30
Total unsaturated fatty acid	73.63	75.80
Total saturated fatty acid	26.19	23.40

ND = Not detectabl .

[A] Values are means ± SD from triplicate determinations.

Values with different superscript letters in the same row indicate significant different ($P < 0.05$).

Vitamin and Phytochemical Contents

Table 4.9 shows the vitamin and phytochemical contents of solvent extracted and cold-pressed SRBO. The results show that cold-pressed SRBO contained significantly higher vitamin B3, vitamin E, biotin, gamma oryzanol, and total phenolic contents than that of hexane extracted SRBO ($P < 0.05$). Cholesterol and vitamin A, B1 and B2 were not found in both SRBO. Our results were in agreement with other literature. Moreau and Kamal-Elddin (2008) reported that cold pressing retained minor compounds such as volatiles, phenolic compounds and chlorophyll but low yield of oil. Vitamin and phytochemical are components that have an amphiphilic structure with a hydrophilic part and a hydrophobic part. They might not dissolve well in hexane during extraction at room temperature. Otherwise, the higher vitamin and phytochemical contents in cold-pressed SRBO may also caused by the lower yield of oil. The higher oil yield may dilute the concentration of vitamin and phytochemical contents in solvent extracted SRBO. However, both solvent extracted and cold-pressed SRBO had high contents of vitamin E, gamma oryzanol, phenolic and flavonoid content. SRBO contained vitamin E, gamma oryzanol, total phenolic and total flavonoid contents with 0.80-0.93 mg/g oil (80-93 mg/100 oil), 17.4-19.0 mg/g oil (1.74-1.90 g/100 g oil), 11.39-14.70 mg FAE/g oil and 6.90-7.54 mg CE/g oil, respectively. Our results were in agreement with other reports. Rice bran contains a significant amount of natural phytochemicals such as oryzanols, tocopherols and tocotrienols that have been reported as the strongest antioxidants in rice bran (Lai *et al*., 2009; Godber and Wells, 1994; Orthoefer and Eastman, 2004). The role of phytochemical as natural antioxidants has attracted considerable interest due to their pharmacological functions. Increased consumption of phenolic compounds has been associated with the reduced risk of cardiovascular diseases and certain cancers (Shen *et al*., 2009). Goffman and Bergman (2004) studied the genotypic and environmental effects of the kernel phenolic content, and found that bran color was highly statistically significant for bran phenolic contents. Flavonoids are one group of phenolics, which consists of two aromatic rings linked by three carbons that are usually in an oxygenated heterocyclic ring (Liu, 2004).

Other phytochemicals in rice bran such as tocols (vitamin E) and gamma oryzanols are also antioxidants. Crude rice bran oil contains 1.5-2.9% gamma oryzanol but during refining 90% of gamma oryzanol goes into the soapstock as refinery waste (Patel and Naik, 2008). Lai *et al*. (2009) reported that rice bran oil from solvent extraction process of Japonica rice bran contained total contents in phenolic compounds, oryzanols, and total tocols of 1.97-1.47 g/100 g extract, 0.98-1.31 g/100 g extract and 21.3-100.7 mg/100 extract, respectively. Mezouari *et al*. (2006) reported that Thai crude rice bran oil contained 50.8 mg/100 g oil of total tocopherol and 1.60 g/100 g oil of gamma oryzanol. Narayana et al. (2002) reported that the crude rice bran from local rice bran oil processing industries in India contained gamma oryzanol at the level of 1.30-1.68 g/100 g oil. Rodrigues and Oliveira (2010) reported that rice bran oil has been cited as an important lipid source with hypocholesterolemic effect. The lowering of cholesterol levels by rice bran oil can be attributed to its high level of unsaponifiable matter that contains a unique complex of antioxidant compounds such as 100–1000 mg/kg of vitamin E (tocopherols and tocotrienols) and 0.9–2.9% of gamma oryzanol.

Table 4.9 Vitamin and phyotochemical contents of Sangyod rice bran oil.

Compositions	Method of extraction	
	Solvent	**Cold pressing**
Vitamin A (mg/g oil)	ND	ND
Vitamin B1 (mg/g oil)	ND	ND
Vitamin B2 (mg/g oil)	ND	ND
Vitamin B3 or Niacin (mg/g oil)	0.020 ± 0.003^{aA}	0.030 ± 0.004^{a}
Vitamin B6 (mg/g oil)	ND	ND
Vitamin E (mg/g oil)	0.80 ± 0.003^{b}	0.93 ± 0.005^{a}
Biotin (µg/g oil)	0.10 ± 0.021^{b}	0.30 ± 0.006^{a}
Cholesterol (mg/g oil)	ND	ND
Gamma oryzanol (mg/g oil)	17.4 ± 0.02^{b}	19.0 ± 0.01^{a}
Total phenolic content (mg FAE /g oil)[B]	11.39 ± 0.36^{b}	14.70 ± 0.37^{a}
Total flavonoid content (mg CE/g oil)[C]	6.90 ± 0.33^{a}	7.54 ± 0.31^{a}

[A] Values are means ± SD from triplicate determinations.

[B] FAE = Ferulic acid equivalent

[C] CE = Catechin equivalent

ND = Not detectable

Values with different superscript letters in the same row indicate significant different ($P < 0.05$).

The Element Contents

The element contents of the solvent extracted and cold-pressed SRBO were different, as shown in Table 4.10. Macro, micro and toxic elements in SRBO were analyzed. Most elements were higher found in cold-pressed SRBO than that of solvent extracted SRBO ($P < 0.05$). Among all elements tested, phosphorus (P) was the dominant trace mineral in both oils. These data indicated that SRBO had a high content of phospholipids, especially in cold-pressed SRBO, containing two times higher content of P than that of solvent extracted SRBO. Crude bran oil tends to have higher levels of phospholipid, which must be removed through the refining process. Rice bran oil is also unique in that it contains higher levels of wax (3-9%) and unsaponifiable components (2-5%) than other vegetable oils, which can cause processing problems but also may contribute to the unique health benefits attributed to rice bran (Godber, 2008). Fe and Cu might contribute to oxidation of SRBO during handling and processing as well as during storage of SRBO products. Our results are in agreement with Mezouari *et al*. (2006). They reported that Thai crude rice bran oil contained Fe and Cu of 2.6 and 0.17 mg/kg oil, respectively. A little amount of Cd was found in both SRBO. Solvent extracted SRBO was absent of As and Cd. However, a little amount of As and Cd were found in the cold pressed SRBO with lower amounts than that of the standard legislation of Thai edible oil. The element contents of rice bran depend on the nutrient availability of the soil in which the crop is grown. Rice bran also contains small quantities of the minerals iron, aluminum, calcium, chlorine, sodium, potassium, magnesium, manganese, phosphorus, silicon, and zinc (Ramezanzadeh *et al*., 2000). The contamination of elements from a polluted environment might result in the accumulated elements in SRBO, which may be associated with nutritive values and quality changes of product.

Table 4.10 Element contents of Sangyod rice bran oil.

Elements contents of SRBO (mg/kg oil)	**Method of extraction**	
	Solvent	**Cold pressing**
Calcium (Ca)	19.20 ± 0.040^{bA}	33.89 ± 0.217^{a}
Phosphorus (P)	65.05 ± 0.083^{b}	125.51 ± 0.127^{a}
Magnesium (Mg)	10.55 ± 0.090^{a}	8.64 ± 0.110^{b}
Potassium (K)	6.72 ± 0.115^{a}	5.93 ± 0.105^{a}
Iron (Fe)	1.42 ± 0.003^{b}	2.08 ± 0.002^{a}
Copper (Cu)	0.46 ± 0.003^{a}	0.34 ± 0.002^{a}
Cadmium (Cd)	0.01 ± 0.000^{a}	0.01 ± 0.001^{a}
Arsenic (As)	ND	0.03 ± 0.002
Lead (Pb)	ND	0.49 ± 0.003

[A] Values are means ± SD from triplicate determinations.

ND = Not detectable

Values with different superscript letters in the same row indicate significant different ($P < 0.05$).

3. Emulsion properties of cold-pressed rice bran oil nano-emulsion stabilized by glyceryl monostearate (GMS)

Formation of cold-pressed rice bran oil nanoemulsion

The purpose of our initial experiments was to establish the maximum amount of cold-pressed rice bran oil (CPRBO), and minimum emulsifier (glyceryl monostearate: GMS), that could be used to prepare stable CPRBO nanoemulsions. The influence of CPRBO and GMS concentration on the particle distribution, mean particle diameter, zeta-potential, creaming index, phytochemical conent, antioxidant activity and color were studied. For study the effect of oil concentration, the ratio of oil:emulsifier was fixed at 10:1 and the concentrations of oil were 10, 20, 30 and 40 wt%. And emulsifier concentration study, 30% of CPRBO with 66-70% aqueous phases containing different concentrations of GMS (1 to 5 wt%) were used for producing emulsion. The dynamic light scattering particle size analyzer measured the particle size distribution and the droplet size of the CPRBO nanoemulsion prepared as shown in Figure4.2-4.3. The data show that the particle size distribution of the nanoemulsions was monomodal (Figure 4.2a and Figure 4.2a). As shown in the Figure 4.2, increasing CPRBO concentration from 10 to 40% led to an increase of the droplet size in the emulsion. However, results using the 30% CPRBO concentration showed a similar pattern to that of the 10% and 20% CPRBO. The emulsion system with high oil concentration (40%) required an increased quantity of hydrophobic emulsifier to disperse the oil completely and for further stabilization. It can also be observed that nanoemulsion with 30% CPRBO and 3% GMS has narrow droplet size distribution compared with other nanoemulsions. The size distribution of the droplets in the emulsion was obtained and corresponding mean size was calculated from the particle size analyzer (d_{32} and d_{43}). The average droplet size of the bran oil emulsion depended on the types and the compositions of the emulsifiers as shown in Figure 4.2 band Figure 4.3b. The mean droplet diameter tended to increase as CPRBO was increased up to 40% (Figure 4.2b). But the mean droplet diameter tended to

decrease as GMS concentration was increased (Figure 4.3b), 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; Walstra 2003; Jafari *et al*., 2008). Figure 4.3b shows that when 30% CPRBO concentration was used, the droplet size of the nanoemulsion prepared with 1- 5% GMS was decreased gradually. However, there were no significantly different of d_{32} and d_{43} when used 3-5% GMS. Therefore, the condition of 30% CPRBO and 3%GMS was used for the production of CPRBO nanoemulsion in the further study. The minimum droplet diameter that could be produced also depended on CPRBO and GMS concentration. In the remainder of the experiments, we prepared CPRBO nanoemulsions using GMS concentrations that were capable of producing small droplet sizes without having too much excess GMS present, that is 3% GMS and 30% CPRBO.

There are a number of possible reasons to account for the observed decrease in droplet size with increasing emulsifier concentration. The amount of droplet surface area can be stabilized by the emulsifier increases and the rate at which the emulsifier adsorbs to the droplet surfaces increases, thereby facilitating droplet disruption and retarding droplet coalescence. The ability of emulsifier to generate repulsive interactions between the oil droplets and to form an interfacial membrane that is resistant to rupture also plays an important role in stabilizing the droplets against flocculation and coalescence during long-term storage. It should be noted that these values were determined under a specific set of homogenization conditions, for example, oil concentration, oil phase composition, aqueous phase composition, temperature, operating pressure, and number of passes. 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.

Zeta-potential is a useful indicator to understand the behavior and interaction of particles and droplets in an aqueous solution, which is affected by factors such as pH, electrolytes and surfactant (Karraker and Radke, 2002). The influence of RBO and GMS concentration on zeta-potential measurement at pH 7 are shown in Figure 4.2b and Figure 4.3b. The results showed that the zeta-potentials of all samples displayed a negative value and were dependent on the surfactant type. Zeta-potential tended to increase as GMS was increased (lower negative zeta-potential) (Figure 4.3b). However, the result showed that CPRBO concentration between 10-40% had no effect on the changes of zeta-potential of CPRBO nanoemulsion (Figure 4.2b). In this study, the nanoemulsion formed with 1-5% GMS showed the zeta-potential value ranging -91.13 to -82.50 mV. The CPRBO nanoemulsion stabilized by GMS indicated larger negative zeta-potential value compared with RBO nanoemulsion stabilized by the whey protein, gum Arabic and modified starch (Chareon *et al*., 2011) and RBO nanoemulsion stabilize by Tween 80 and Span 80 (Nguyen *et al*., 2012) In the dispersion system, particles or droplets have charges on their surface because of the selective adsorption of ions, including protons (Lin and Chaudhury, 2008).

Color is one of the major attributes which affects the consumer perception of quality and it determines the purchase and regular consumption. It can also be used as a direct quality estimate of fruits, beverages, oils, and even non-dairy emulsions. The effects of CPRBO and GMS concentration on color of rice bran oil-in-water emulsions are showed in Figure 4.4. The data showed that the increase of CPRBO concentration caused the increase in a* value and b* value but decrease in L* values (Figure 4.4a). We also found that lightness (L*) and yellowness (b*) of CPRBO emulsion tended to decrease as GMS concentration was increased Figure 4.4a). A large decrease in L* values of CPRBO mampoemulsion was observed when the GMS concentration was increased from 2 to 5%. As the average droplet size of CPRBO nanoemulsion decreased (Figure 4.2 and 4.3), the L* values decreased. Chantrapornchai *et al*. (1998) reported that as the droplet size increases, the scattering efficiency of the droplets decreases, which causes a reduction in lightness (lower L value) and an enhancement of color. In fact, absorption of

the light wave by the emulsion is largely responsible for 'chromaticness' (blueness, greenness, redness, etc.) which is strongly related to a* and b* values (McClements, 1998).

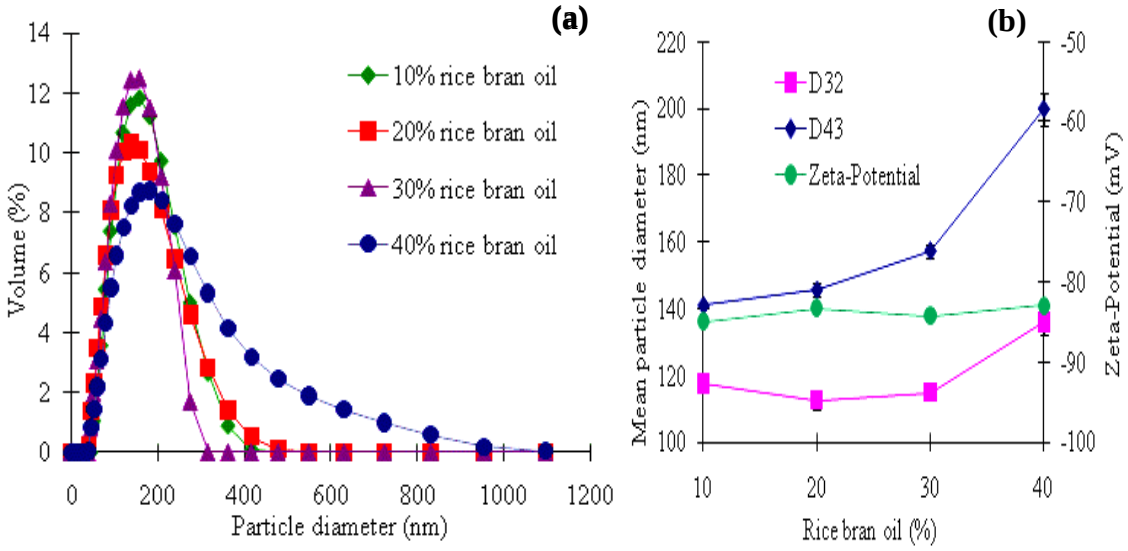

Figure 4.2 Influence of cold-pressed rice bran oil concentration on the particle diameter, mean particle diameter and zeta potential of diluted cold-pressed rice bran oil nanoemulsions stabilized by 3% glyceryl monostearate (GMS).

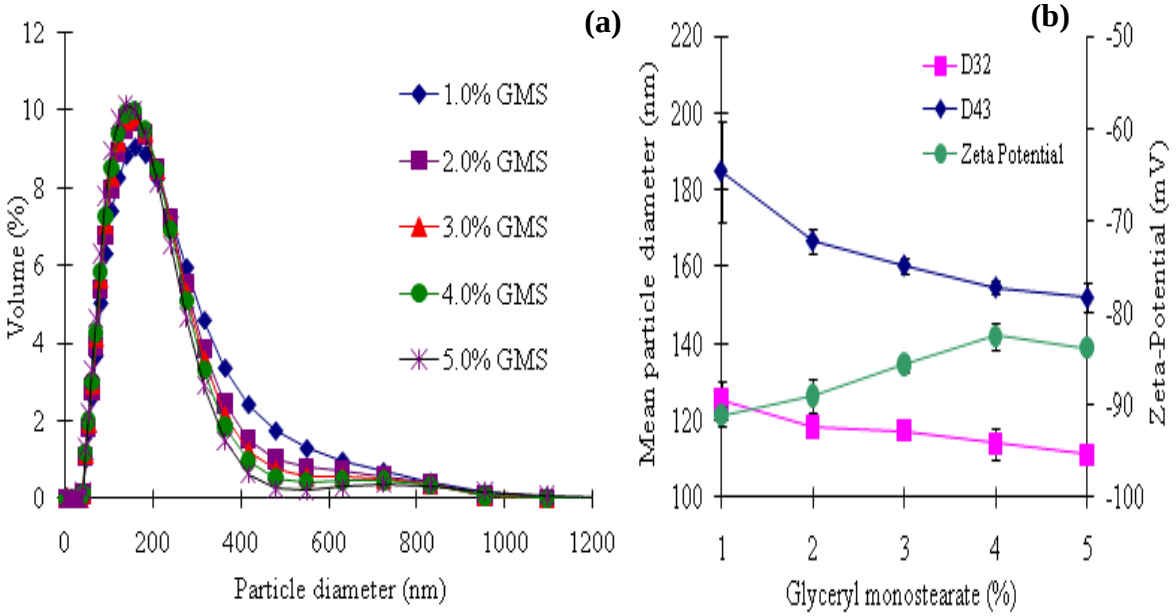

Figure 4.3 Influence of glyceryl monostearate (GMS) concentration on the particle diameter (a), mean particle diameter and zeta potential (b) of diluted 30% cold-pressed rice bran oil nanoemulsions.

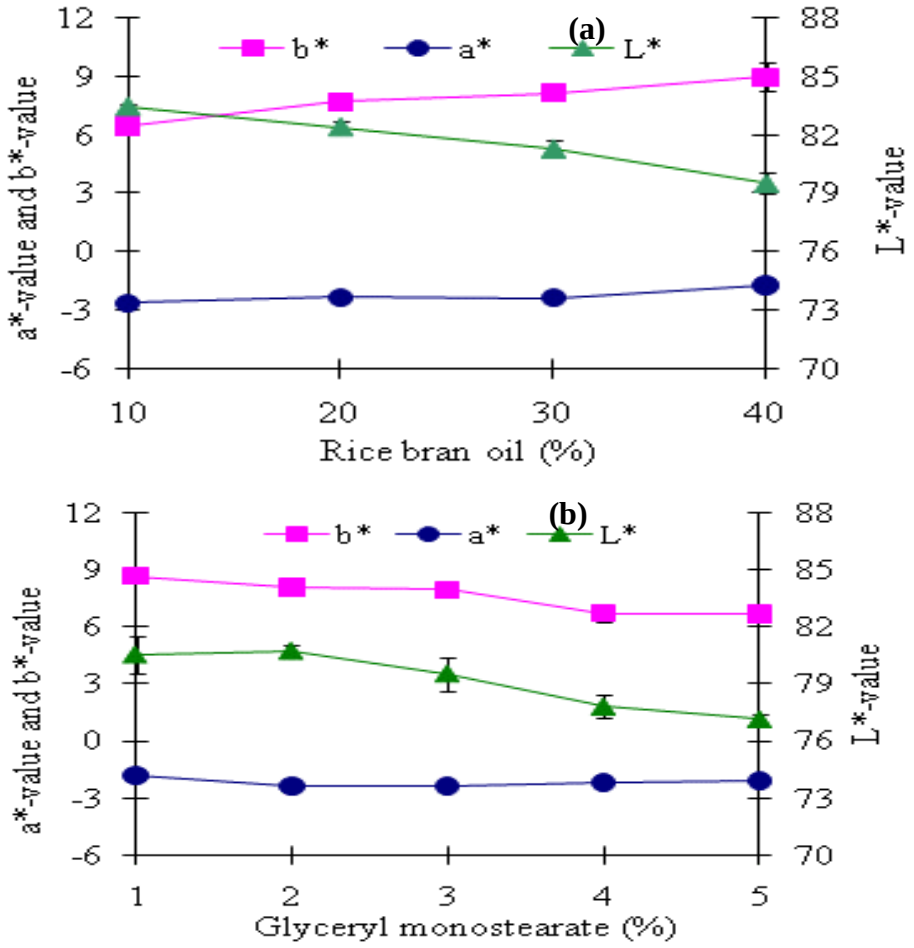

Figure 4.4 Influence of cold-pressed rice bran oil concentration (a) and glyceryl monostearate concentration (b) on color of cold-pressed rice bran oil nanoemulsions.

Antioxidant property and oxidative stability of cold-pressed rice bran oil nanoemulsion

Rice bran contains a significant amount of natural phytochemicals such as oryzanols, tocopherols and tocotrienols that have been reported as the strongest antioxidants in rice bran (Godber and Wells, 1994; Lai *et al*., 2009; Orthoefer and Eastman, 2004). Our previous work found that CPRBO contained the total phenolic content ranging from 11.59 to 11.27 mg FAE/g oil and gamma oryzanol content ranging from 2.03 to 2.25 g/100 g oil (Thanonkaew *et al*., 2012). In this work the effects of CPRBO and GMS concentration on phytochemical contents and

antioxidant activity of CPRBO nanoemulsions were studied (Figure 4.4). The antioxidant activities of CPRBO nanoemulsion were evaluated by using DPPH assay. We found that the increase of CRBO concentration cause the increase in the phytochemical contents and antioxidant activity of CPRBO nanoemulsions. However, the increase of the GMS concentration had no impact on antioxidant activity, gamma oryzanol and total phenolic compound content of CPRBO nanoemulsion. The data replied that the high phytochemical content in CPRBO could improve the phytochemical contents and antioxidant activity of CPRBO nanoemulsions.

Recently, food manufacturers have been interested in utilization of the polishing by-products such as rice bran. Rice bran oil contains well-balanced saturated and unsaturated fats and provides a good source of vitamin E, antioxidants, oryzanol and other micronutrients. However, food application of rice bran oil is limited because of lipid oxidation. One of the promising technologies is nanoemulsion fabrication, which is being applied to enhance the oral bioavailability and oxidative stability of the poorly water-soluble bran oils. Emulsion-based delivery systems are finding increasing utilization in the food industry to encapsulate and protect polyunsaturated fats from oxidation. This study carried out to determine the oxidative stability of RBO nanoemulsions prepared using 30% CPRBO and 3% GMS in 10 mM phosphate buffer pH 7 during storage at 25 ℃ for 90 days. The oxidative stability was monitored by measuring hydroperoxide and TBARS concentration. Hydroperoxide is an indicator the formation of primary reaction product and TBARS is an indicator of the formation of secondary reaction products. Many studies have found good correlations between hydroperoxide and/or TBARS and other secondary reaction product markers of lipid oxidation in emulsions and bulk oils, such as specific aldehydes. Changes of lipid oxidation, phytochemical contents and antioxidant activity of CPRBO nanoemulsions during storage for 90 days are shown in Figure 4.6a. There was a notable increase in Hydroperoxide and TBARS after 30 days storage followed by a gradual increase at longer times, indicating that an appreciable amount of lipid oxidation had occurred in CPRBO

nanoemulsion. However, the increase of gamma oryzanol, phenolic content and antioxidant property of CPRBO nanoemulsions was found after 60 days of storage (Figure 4.6b).

Nanoemulsion, which has increased specific surface area, may not be considered a good system to retard lipid oxidation. However, surface characteristics of the droplets are more important than their size. Several authors have found that surface charge can influence the oxidative stability of lipid droplets in emulsion systems (Yoshida and Niki 1992; Mei *et al*., 1998). Interfacial properties of the lipid droplets could be the important determinants of the degree of oxidation in an emulsion. The physical characteristics of the droplets may also affect the oxidative stability of o/w emulsion depending on their concentration, size, physical state and charge (Mancuso *et al*., 1999). There are several reports that the effect of droplet size on lipid oxidation is not uniform; some studies indicated that smaller droplet sizes led to higher oxidation rates because of increased surface area (Lee et al. 2009), but another reported no dependence of lipid oxidation rate on droplet size (Roozen *et al*., 1994). Because limited amounts of hydroperoxides were available in the systems, they might have all been present at the droplet surface in every o/w emulsion system studied.

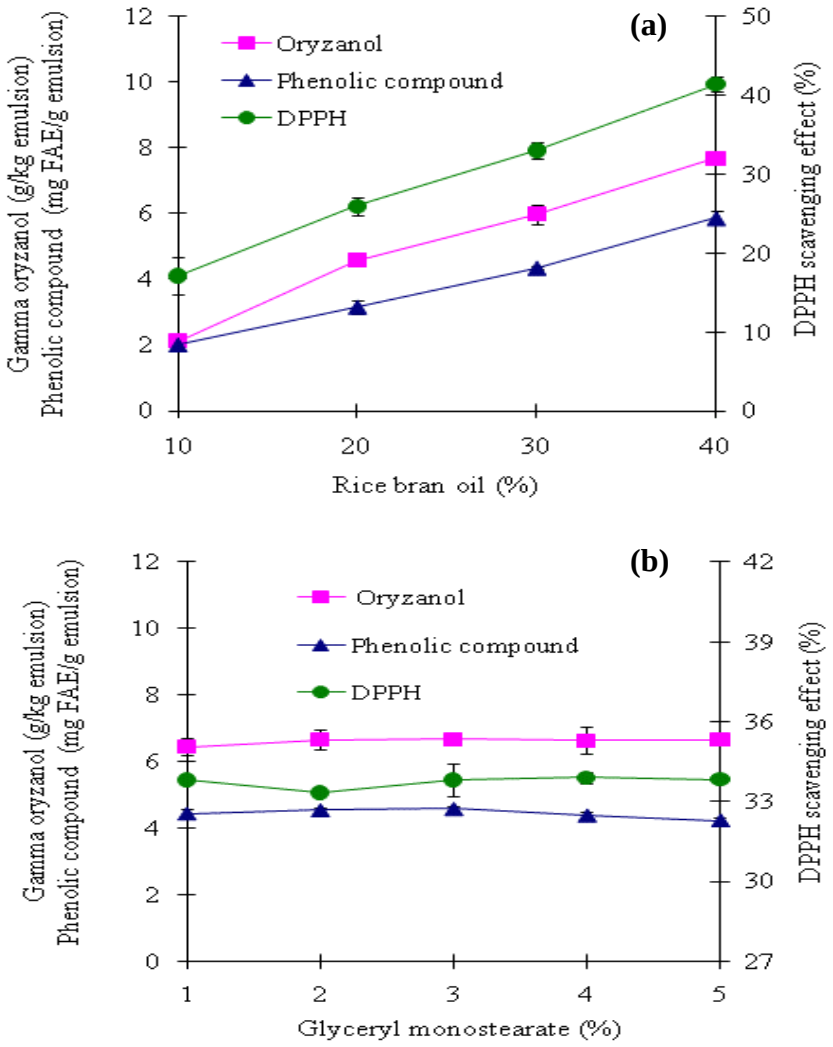

Figure 4.5 Influence of cold-pressed rice bran oil concentration (a) and glyceryl monostearate concentration (b) on phytochemical contents and antioxidant activity of cold-pressed rice bran oil nanoemulsions.

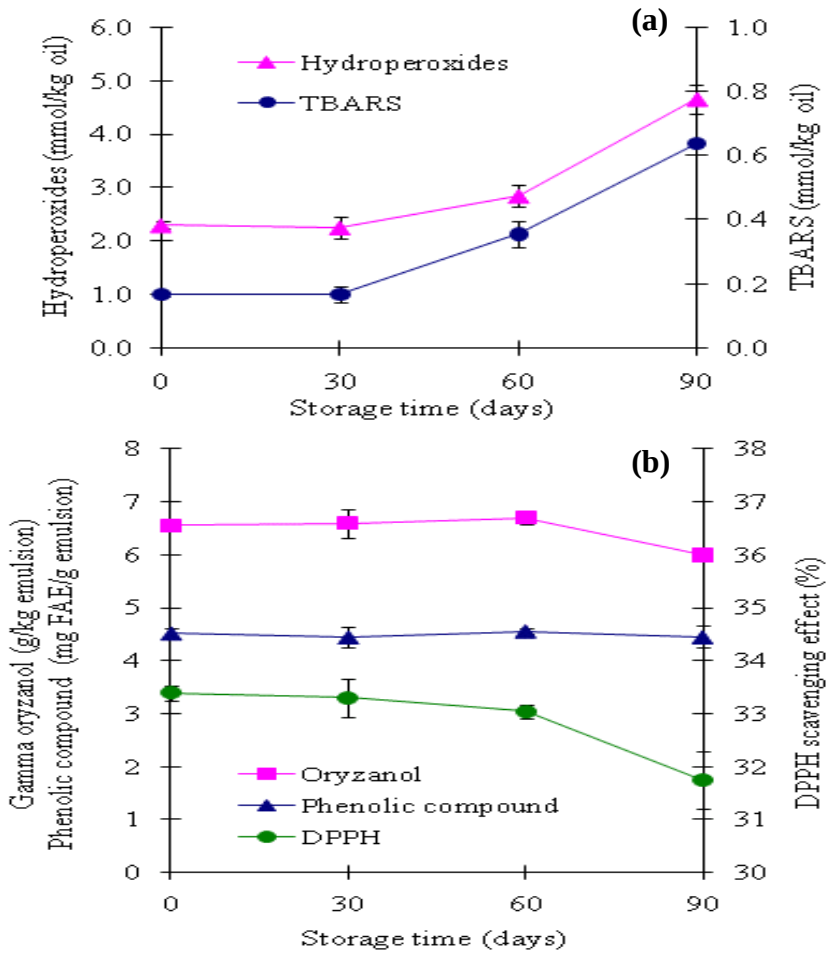

Figure 4.6 Changes of lipid oxidation, phytochemical contents and antioxidant activity of cold-pressed rice bran oil nanoemulsion during storage at 25 °C for 90 days.

Influence of the environmental stress on physicochemical properties of CPRBO nanoemulsions of cold-pressed rice bran oil nanoemulsion

The pH of the aqueous phase in emulsified foods and beverages may vary considerably depending on the nature of the product, e.g., acidic in soft drinks and neutral in infant formula. In this section, we therefore examined the influence of pH on the physicochemical properties of CPRBO nanoemulsion. The pH dependence of the mean particle diameters (d32 and d43) of CPRBO nanoemulsion is shown in Figure 4.7a. The nanoemulsions remained relatively stable

to droplet aggregation (d32 ≈ 115-121 nm and d43 ≈165-185 nm) at high pH values (pH 7 to 8). On the other hand, a large increase in mean particle diameter was observed at low pH value (pH 6-2). Particle size distribution measurements provided additional insights into the nature of the droplet aggregation in the emulsions (Figure 4.7b). At relatively low pH values (pH 4-2) (d32 ≈ 18.48-112.771 μm and d43 ≈65.19-217.65 μm), a large population of highly aggregated droplets was observed. These results indicate that CPRBO nanoemulsion were not stable at low pH. The pH dependence of the ζ-potential for the nanoemulsions is compared in Figure 4.7a. The ζ -potentials of the CPRBO nanoemulsion coated by GMS was negative at all pH values, which can be attributed to the presence of some negatively charged side groups. The ζ -potential of CPRBO nanoemulsion went from highly negative at high pH to nearly zero charge at low pH. The presence of a thick emulsifier layer may also decrease the magnitude of the attractive van der Waals forces acting between the droplets, which also increases emulsion stability to flocculation (Guzey and McClements 2007). This may have important consequences for the interactions of GMS-coated 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 promote lipid oxidation (McClements and Decker 2000; Hu *et al*., 2003). The influence of pH on the changes in color of CPRBO nanoemulsions is shown in Figure 4.7c. The data showed that pH 5-2 had a very high impact on b* and L* values but it was slightly impact on a* value. At low pH (pH 5-2), it also caused the chages of appearance viscosity of CPRBO nanoemulsion (Figure 4.7d). Visual observations of CPRBO nanoemulsions showed very different patterns of phase separation at pH values between pH 5-2 (Figure4.8b). At pH 4-2, we observed a distinct cream layer on top of the nanoemulsion and a transparent serum layer at the bottom. At pH 5, a cream layer was still observed, but the serum layer was slightly turbid, indicating that droplet aggregation was less extensive at this pH. Interestingly, a cream was not formed at the top of the nanoemulsions at pH 6-7. At pH 6-9, phase separation was

not observed, but the transparency of the samples was significantly reduced, indicating that the droplet aggregation that occurred at this pH was not sufficient to cause phase separation.

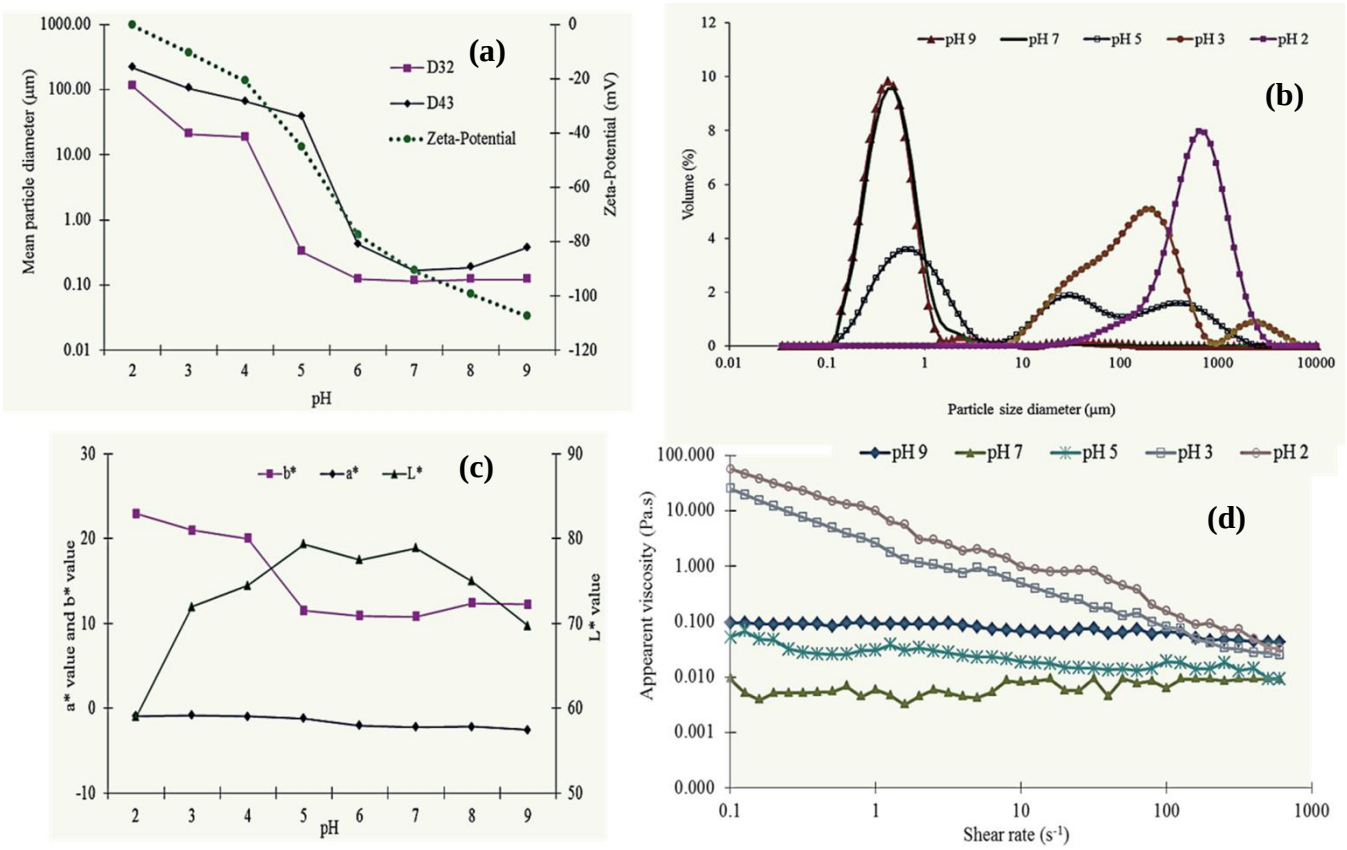

Figure 4.7 Influence of pH on physicochemical properties of cold-pressed rice bran oil nanoemulsions.

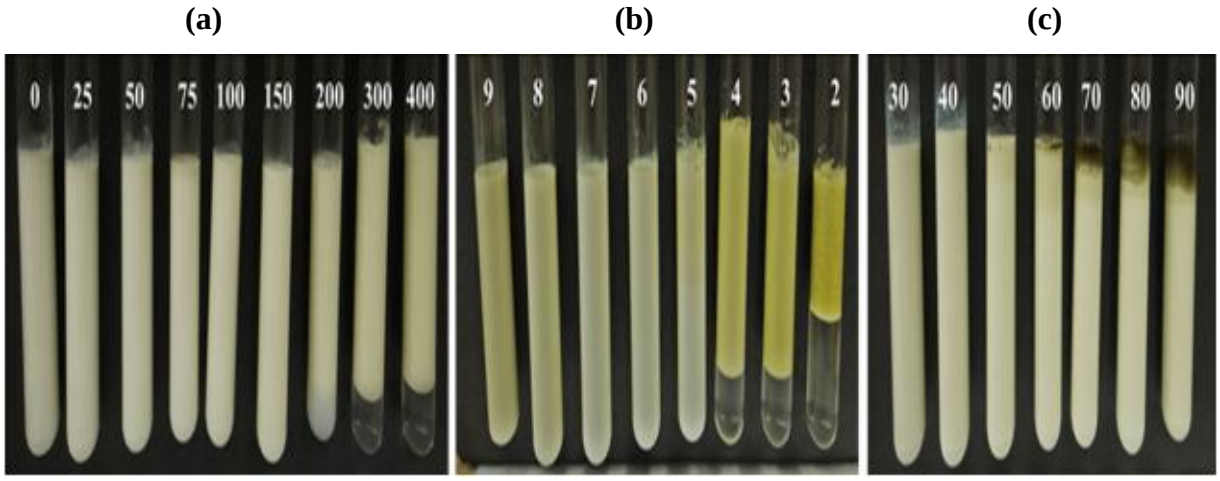

Figure 4.8 Influence of salt, pH and heat on the creaming index of cold-pressed rice bran oil nanoemulsions.

The ionic strengths of emulsified foods and beverages vary considerably depending on the nature of products. It is usually important that emulsions remain stable to droplet aggregation and creaming in the presence of salts. We therefore examined the influence of ionic strength (0-400 mM NaCl) on physicochemical properties of nanoemulsions at pH 7 (Figure 4.9). The particle size of emulsion samples containing different salt levels was measured after they were stored for 1 day at ambient temperature (≈25 °C) (Figure 4.9a). In the absence of salt, the mean droplet diameters (d32 and d43) of the CPRBO nanoemulsions were initially approximately 115 nm and 162 nm, respectively. There was no significant difference in the particle size distribution or mean particle diameters of nanoemulsions containing different levels of salt at 0-50 mM. On the other hand, an appreciable increase in mean particle diameter (from d32 = 121 to 7697 nm and d43 = 185 to 21732 nm) was observed in the CPRBO nanoemulsions when the salt concentration was increased from 75 to 400 mM (Figure 4.9a). The particle size distribution data indicated that the majority of droplets in these nanoemulsions remained stable to aggregation and that a high population of large particles was present at high salt concentration (Figure 4.9b). This work in agree with Chareon *et al*. (2011). They reported that the RBO

emulsion was an appreciable increase in the mean particle diameter of the whey protein isolated-stabilized emulsions at ionic strengths of 200 mM and higher. CPRBO nanoemulsions was a decrease in the magnitude of the ζ-potential with increasing salt concentration (Figure 4.9a), which can be attributed to electrostatic screening effects. Counter ions in the aqueous phase tend to associate with the negatively charged group on the emulsifier surface due to electrostatic attraction, thereby reducing the net charge (Demetriades *et al*., 1997). This increase in droplet aggregation at higher salt concentrations is due to screening of the electrostatic repulsion between the emulsifier-coated droplets (McClements 2005; Guzey and McClements 2007). Above a critical salt level, the electrostatic repulsion is no longer strong enough to overcome the attractive interactions acting between the droplets (van der Waals and hydrophobic). These particles may not have been detected in some of the emulsions due to the fact that they quickly moved to the top of the samples due to their large size. Consequently, there may have been problems removing representative samples from the original emulsions or due to rapid creaming of these particles within the particle size measurement chamber. The influence of ionic strength on color of CPRBO also studied (Figure 4.9c). We found that the increase of salt concentration (100-400 mM) caused the decrease in L* value and increase b* value. It also caused a high viscosity of CRBO nanoemulsion (Figure 4.9d). Visual observations of the emulsions containing different salt levels indicated that a distinct cream layer formed on top of nanoemulsions at higher salt levels (≥200 mM), but that the rest of the emulsions were relatively stable to gravitational separation (Figure 4.8a). In addition, visible observation of the conventional emulsions containing high salt levels indicated that they remained optically opaque throughout but that a small amount of aggregated droplets (white material) was present on their surfaces.

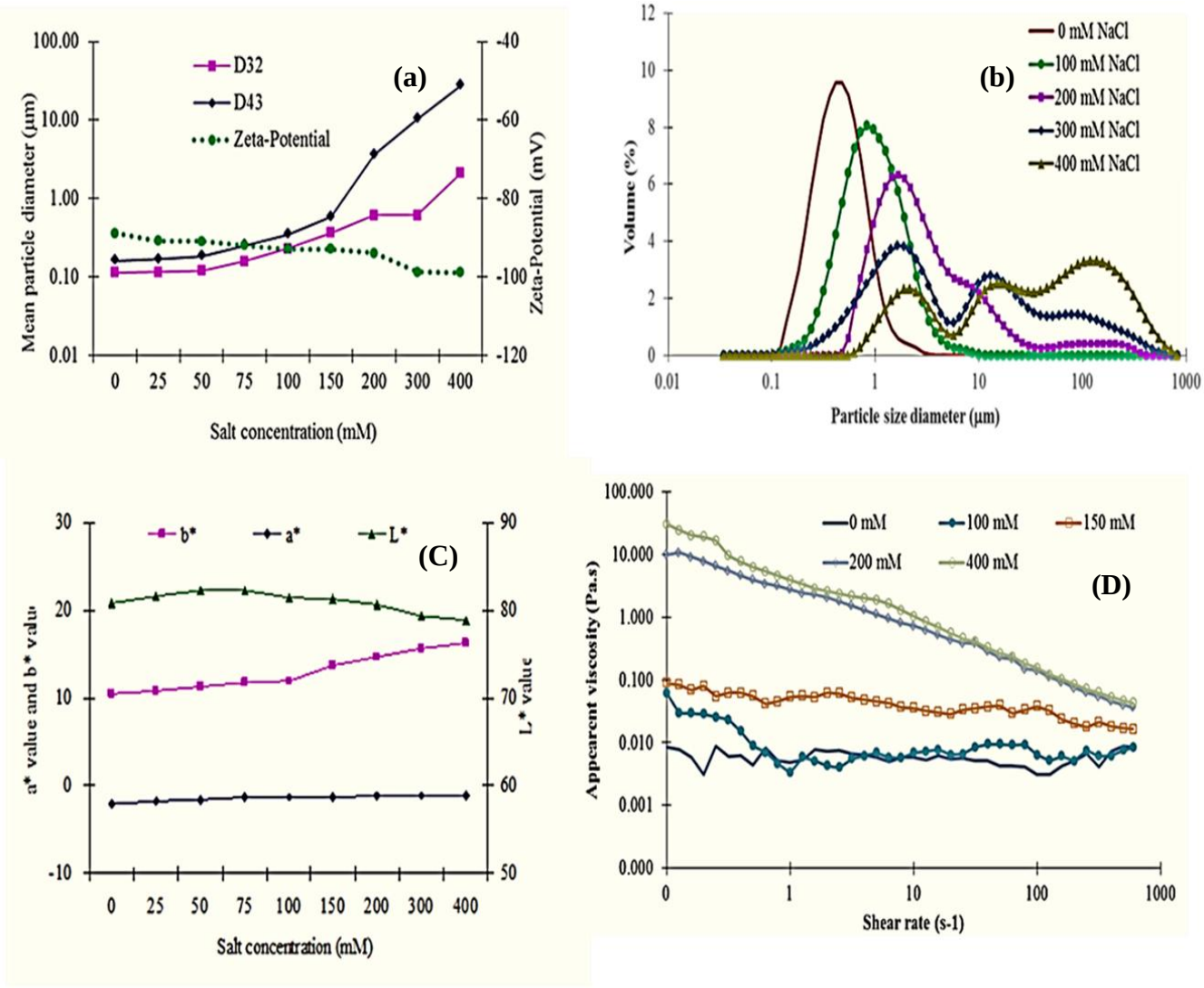

Figure 4.9 Influence of salt concentration on physicochemical properties of cold-pressed rice bran oil nanoemulsions.

Emulsified food and beverage products often experience variations in their temperature during manufacture, storage, and utilization, and so we examined the influence of thermal processing on physicochemical properties of CPRBO nanoemulsion (Figure 4.10). CPRBO nanoemulsions were heated in the presence of 50 mM NaCl, from 30 to 90 °C for 30 min at pH 7, and then stored for 1 day at 25 °C prior to analysis. 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 (Kim *et al*., 2002; Kim *et al*., 2005). The mean particle diameters of the nanoemulsions were then measured. In the absence of added salt, nanoemulsions were stable to droplet aggregation after heating at 90 °C for 30 min at pH 7 (data not shown). Previous study has also shown that whey protein-stabilized emulsions are stable to droplet aggregation when heated in the absence of salt at neutral pH due to the strong electrostatic repulsion between them (Kim *et al*., 2002). Chareon *et al*. (2011) also reported that in the absence of added salt, the RBO emulsions stabilized with whey protein, gum Arabic and modified starch 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, with the exception of the modified starch sample at high temperatures. The addition of 50 mM NaCl prior to heating had a strong destabilizing effect on nanoemulsions. The nanoemulsions were relatively stable to droplet aggregation at temperatures below 50 °C with no pronounced increase in mean particle diameter. However, the mean particle diameter of the nanoemulsions containing salt did increase at higher holding temperatures (50 to 90 °C). The electrical characteristics of CPRBO nanoemulsions were unchanged by heating. 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). The increase in droplet aggregation at high temperatures can be attributed to an increase in the surface hydrophobicity of the droplets due to emulsifier unfolding, which leads to a strong hydrophobic attraction between the droplets (Monahan *et al*., 1996). The overall

attractive force was then presumably strong enough to overcome the net repulsive force, such as steric and electrostatic forces. The studies on the influence of thermal process on color and viscosity of CPRBO nanoemusion are shown in figure 4.9c and 4.9d. We found that the heating did not significantly effect on the color and viscosity of CPRBO nanoemulsion. Visual observation of the nanoemulsions indicated that they remained fairly transparent at holding temperatures less than 60 °C but became increasingly turbid at higher temperatures (70-90 °C) (Figure 4.8c).

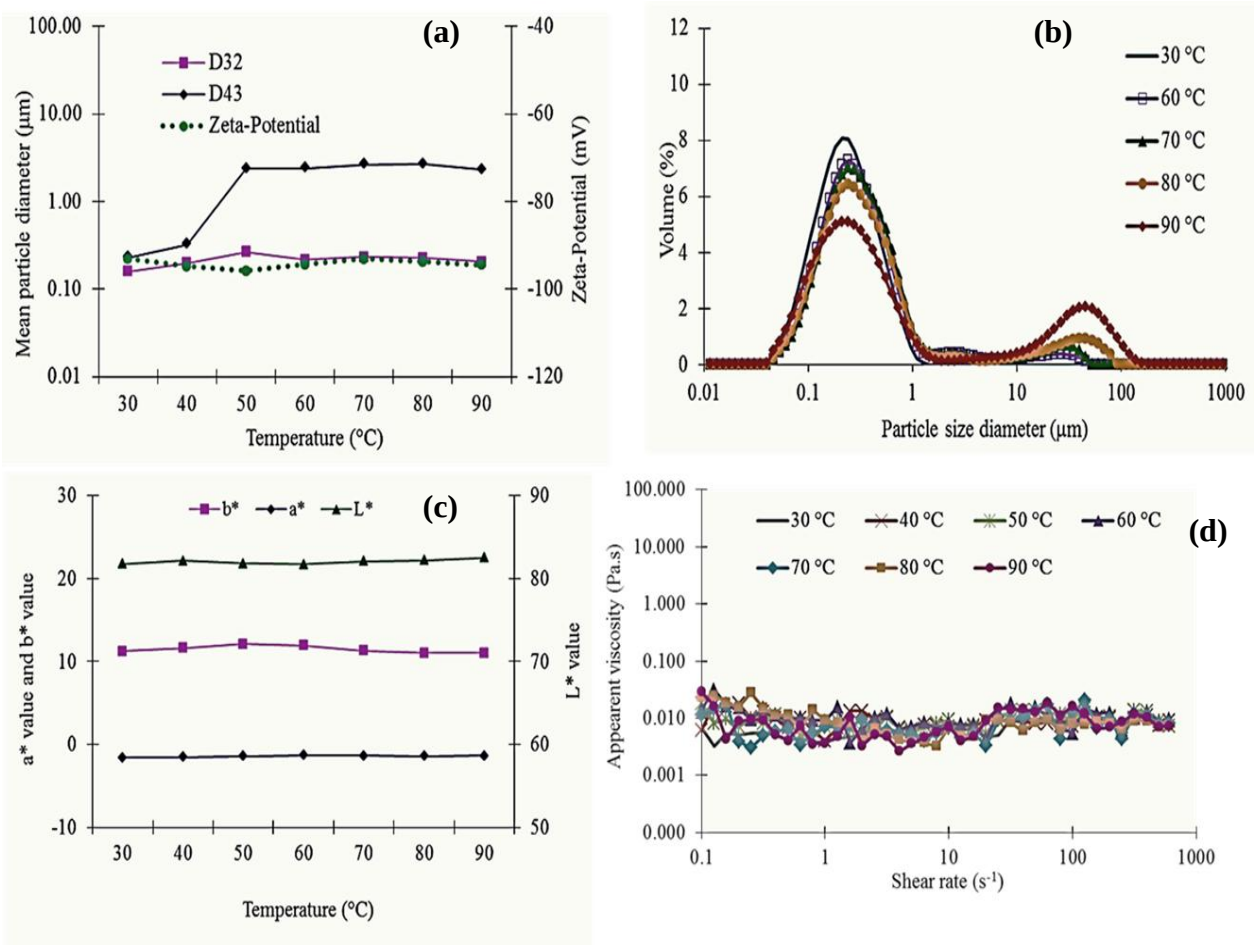

Figure 4.10 Influence of heat on physicochemical properties of cold-pressed rice bran oil nanoemulsions.