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*Original Article*

# Physical, chemical and microbiological properties of mixed hydrogenated palm kernel oil and cold-pressed rice bran oil as ingredients in non-dairy creamer

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**Abstract**

The physical, chemical and microbiological properties of hydrogenated palm kernel oil (PKO) and cold-pressed rice bran oil (RBO) as ingredients in the production of liquid and powdered non-dairy creamer (coffee whitener) were studied. The mixing ratios between hydrogenated PKO and cold-pressed RBO were statistically designed as 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100. The color and viscosity of the mixtures were investigated. As the ratio of cold-pressed RBO increased, the color became darker (L*of 93.06 to 86.25) while the viscosity of the mixtures of 20:80, 10:90 and 0:100 (54 cp.) were the highest amongst the ratios tested. The hydrogenated PKO and cold-pressed RBO mixtures were further chemically tested for fatty acids, γ-oryzanol, α-tocopherol, trans-fat contents and antioxidant activity. There were 10 fatty acids present in hydrogenated PKO with saturated fatty acids being predominant. By contrast, there were only 5 fatty acids found in cold-pressed RBO with monounsaturated fatty acid being the major fatty acid. γ-Oryzanol and α-tocopherol contents were higher with increasing cold-pressed RBO from 0-100% (0 to 1,155.00 mg/100 g oil and 0.09 to 30.82 mg/100 g oil, respectively). Antioxidant activity was increased with increasing cold-pressed RBO from 0-100% (9.26 to 94.24%). The pure hydrogenated PKO contained higher trans-fat content than that of the 90:10 and 80:20 mixtures (2.73, 1.93 and 1.85 mg/100 g oil, respectively) while no trans-fat was detected in other samples. Therefore, substitution of hydrogenated PKO by cold-pressed RBO from 30-100% would offer more nutritional value.

**Keywords:** cold-pressed RBO, hydrogenated PKO, γ-oryzanol, fatty acids, antioxidant activity

## 1. Introduction

Palm kernel oil (PKO), extracted from the kernel of palm fruit, is rich in saturated fatty acids namely lauric acid (C12:0) and other major fatty acids such as myristic (C14:0) and oleic acids (C18:1) (Rossell *et al*., 1985; Nik Norulaini *et al*., 2004; Alamu *et al*., 2008; Kok *et al*., 2011). This fatty acid profile gives PKO a solid consistency at cool ambient temperatures below 30°C (Rossell, 1985). PKO is a widely used ingredient for production of non-dairy creamer (Kelly, 1999).

Hydrogenation is the process whereby hydrogen is chemically added to react with the unsaturated double bonds present in fatty acids in order to prolong shelf life of the oils (Xiao, 2007) and to expand the application of vegetable oil in foods (Jang *et al*., 2005), particularly in the fat industry because of its wide applications to produce margarine, shortenings, frying oils (Murzin and Simakova, 2008), non-dairy products and coffee whitener (Goh, 1994). Vegetable fat or oil is generally used as an essential ingredient in coffee whiteners, for it provides whitening powder, body and viscosity (Melachouris *et al*., 1994). Melnychyn *et al*. (1973) and Tonner *et al*. (1978) used hydrogenated coconut oil as an ingredient to produce coffee whitener while Campbell *et al*. (1992) and Brown *et al*. (2011) used palm kernel oil and soybean oil, respectively. The hydrogenation process changes liquid oils into semi-solid or solid substances (depending on degree of hydrogenation), which have desired melting characteristics and increased stability (Pintauro *et al*.,

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2005). As hydrogenated oil contains mostly saturated fatty acids, it is more stable and does not become rancid as quickly as unhydrogenated oil. However, the hydrogenation process also changes cis-isomer to trans-isomer in the oils. This trans-fat is reportedly a contributor to heart disease and cancer (Mensink and Kata, 1990; Ascherio *et al*., 1999; Fernendez *et al*., 2007). Furthermore, saturated fatty acids also contribute to high cholesterol levels in humans, heart disease and cancer (Yi *et al*., 2011). The substitution of a more nutritious oil is therefore desirable.

Rice bran oil (RBO), particularly cold-pressed RBO (Singanusong and Noitup, 2012) is a naturally rich source of valuable bioactive phytochemicals, most of which show promising significance in nutrition, pharmacy and cosmetics (Da Silva *et al*., 2005; Danielski *et al*., 2005). RBO is the oil extracted from the germ and inner hull of rice. It is notable for its high heat tolerance or smoke point of 213°C (415°F) reported by Watanapoon (2004) and 475°F or higher reported by Patel and Naik (2004). It has a mild flavor, making it suitable for high-temperature cooking methods such as stir frying and deep frying without thickening, smoking, foaming or breaking down (Patel and Naik, 2004). The bioactive phytochemicals in rice bran are the naturally-occurring antioxidants including tocopherols, tocotrienols, γ-oryzanol, lecithin and carotenoids (Chen and Bergman, 2005; Patel and Naik, 2004; Stoggl *et al.*, 2005), flavone tricin (Devi and Arumughan, 2007) and α-octacosanol and squalene (Ha *et al*., 2006). The concentrations of tocopherols, tocotrienols (0.10–0.14%) and γ-oryzanol (0.9–2.9%) in RBO vary largely upon genetic and environmental factors (Diack and Saska, 1994; Lloyd *et al*., 2000; Patel and Naik, 2004).

The antioxidants of RBO have a potential use as additives to improve the storage stability of foods (Nanua *et al*., 2000; Kim and Gerber, 2001; Chen and Bergman, 2005). γ-Oryzanol is a substance found in high quantity only in RBO, and it has the potential to reduce LDL cholesterol (Lichenstein *et al*., 1994; Gerhardt and Gallo, 1998), protect from chronic disease caused by high cholesterol levels (Seetharamasah and Chandrasekhara, 1989), inhibit platelet aggregation (Kaimal, 1999; Eitenmiller, 1997 as cited in Bucci *et al*., 2003), prevent coronary artery disease (Imsanguan *et al*., 2008), protect the skin from the sun burning and prevent wrinkles of the skin (Graf, 1992).

RBO has a very good balance in its fatty acid composition (Ghosh, 2007). Moreover, it is rich in essential fatty acids; linoleic acid (32-38%) and linolenic acid (1-2%). The fatty acid composition of RBO is mainly monounsaturated fatty acid (MUFA), accounting for 40% of total fat. This MUFA has been reported to reduce LDL cholesterol and increase HDL cholesterol (Ghosh, 2007).

Non-dairy creamer is the product that does not make from milk and has other fats than cream as ingredients or creamer that contains cream less than 30% (Ministry of Public Health, 2000). Most of non-dairy creamer is made from coconut and palm kernel oil, available as powdered, liquid and frozen forms (Herbst, 1995) and has the major role to reduce color of coffee and tea and provides flavor (Gardiner, 1977).

The substitution of hydrogenated PKO with cold-pressed RBO for the production of non-dairy creamer or coffee whitener could potentially provide the product with better and balanced nutritional properties and antioxidant activity. This is because cold-pressed RBO not only is rich in unsaturated fatty acids but also contains γ-oryzanol, tocopherols, tocotrienols which would also help to prolong shelf life of the product. The aim of this study was to investigate the properties of different mixing ratios between hydrogenated PKO and cold-pressed RBO.

## 2. Materials and Methods

### 2.1 Materials

Hydrogenated PKO and cold-pressed RBO were kindly supplied by Korn Thai Co., Ltd., Ratchaburi province, Thailand and Kiatsiri Pharmacy, Lopburi province, Thailand, respectively. The extraction of cold-pressed RBO followed the method described by Singanusong *et al*. (2010). Newly milled fine rice bran was pressed for crude oil using the cold press, screw type expeller with 1 hp motor. The crude oil was then filtered through a strainer with 1 mm in diameter, Whatman filter paper no.91, 1 mm filter with vacuum and then passed through a glass tube curling around 30 cm long, 50 watts UV lamp before passing through a magnetic field using permanent magnet for 4 h. Finally, cold-pressed RBO with the temperature lower than 60°C was obtained. Both hydrogenated PKO and cold-pressed RBO were kept in the refrigerator (4-8°C) before analysis. All other reagents and solvents were of analytical grade.

### 2.2 Degumming of cold-pressed RBO

For cold-pressed RBO, an additional degumming step was needed before application. Briefly, 500 g cold-pressed RBO was mixed with 25 ml distilled water in a beaker and stirred for 20 min. The mixed sample was heated until reaching temperature of 80°C and then allowed to cool down to room temperature before centrifugation at 10,000 rpm for 1 h at 20°C (Chomyong, 2008; Singanusong and Noitup, 2009). The clear supernatant (cold-pressed, de-gummed RBO) was collected for further utilization.

### 2.3 Preparation of the mixtures of hydrogenated PKO and cold-pressed RBO

The mixing ratios between hydrogenated PKO and cold-pressed RBO were 100:0 (pure hydrogenated PKO), 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90 and 0:100 (pure cold-pressed RBO) v/v. The mixtures were prepared by completely melting the hydrogenated PKO at 60-70°C. After standing at room temperature for 5 min, hydrogenated PKO was mixed with cold-pressed RBO at the

above mixing ratios and stirred by a magnetic bar at a medium speed for 5 min before analyzing.

### 2.4 Determination of viscosity and color

Viscosity was determined by Brookfield VISCOMETER model DV III RHEOMETER S/N R40020E (Scientific Promotion Co., Ltd., Switzerland). Color (L*, a* and b*) was determined by Color Reader model CR-10 (Konica Minolta Sensing Inc., Osaka, Japan).

### 2.5 Determination of fatty acids, γ-oryzanol, trans-fat and α-tocopherol

Fatty acid profile and trans-fat contents were determined according to the modified methods of the Compendium of Methods for Food Analysis (2003); γ-oryzanol content was determined according to the modified methods of Azrina *et al*. (2008); and α-tocopherol content was determined according to the modified methods of Qiana and Sheng (1998).

### 2.6 Statistical analysis

The experimental design used was a Completely Randomized Design (CRD). All treatments were analyzed in duplicates. Data were statistically analyzed by ANOVA. Differences in means were analyzed using Duncan's Multiple Range Test (DMRT) at 95% confidence level.

## 3. Results and Discussion

### 3.1 Viscosity and color of the oils and blends

The properties of cold-pressed RBO were determined after the degumming step. Degumming is to remove natural gums present in the oil which could hinder their utilization in food products (Singanusong *et al*., 2010). The viscosity and color of hydrogenated PKO, cold-pressed RBO and their mixtures are shown in Table 1. The viscosities of the hydrogenated PKO:cold-pressed RBO mixtures of 100:0, 90:10, 80:20, 70:30 and 60:40 were 46 cp. ($P>0.05$) whereas the hydrogenated PKO:cold-pressed RBO mixtures of 50:50, 40:60 and 30:70 were 50 cp. ($P>0.05$). The samples with mixing ratios of hydrogenated PKO:cold-pressed RBO at 20:80, 10:90 and 0:100 had the highest viscosity (54 cp.) ($P\leq0.05$). The results indicated that the viscosity of the mixtures was significantly increased ($P\leq0.05$) with increasing ratios of cold-pressed RBO. This could be due mainly to cold-pressed RBO still having some gum and wax which contributed to high viscous mixed oil (Hoed *et al*., 2010). In addition, as PKO had already been industrially degummed, dewaxed and refined, therefore, had low viscosity. Therefore, increasing ratio of cold-pressed RBO would result in increasing viscosity. The viscosity of the mixtures and even the pure cold-pressed RBO in this study was much lower than that found in cold-pressed RBO (78.60 cp.) reported by Jennings and Akoh (2010). This might be due to differences in measurement conditions, extraction and genetic and environmental factors.

The desirable range of viscosity of the oil for the production of non-dairy creamer can be varied depending upon types of oil used. As the formula of non-dairy creamer involves using of many ingredients including emulsifiers, texturizing agents, milk powder, sodium casienate, glucose syrup, appropriate synthetic color, flavor and water, the viscosity of the slurry can be adjusted appropriately before being spray dried.

Hydrogenated PKO was transparent light yellow while cold-pressed RBO was dark brown in color. This was mainly due to hydrogenated PKO had been through the

Table 1. Viscosity and color of hydrogenated PKO, cold-pressed RBO and their mixtures.

| PKO:RBO | Viscosity (cp.) | Color | | |
|---|---|---|---|---|
| | | L* | a* | b* |
| 100:0 | $46^{c}\pm1.14$ | $93.06^{a}\pm0.01$ | $1.34^{a}\pm0.05$ | $4.26^{k}\pm0.02$ |
| 90:10 | $46^{c}\pm1.75$ | $92.12^{b}\pm0.03$ | $0.80^{b}\pm0.02$ | $6.47^{j}\pm0.04$ |
| 80:20 | $46^{c}\pm1.10$ | $91.22^{c}\pm0.12$ | $0.23^{c}\pm0.01$ | $9.14^{i}\pm0.01$ |
| 70:30 | $46^{c}\pm1.36$ | $90.37^{d}\pm0.02$ | $0.01^{d}\pm0.01$ | $10.87^{h}\pm0.02$ |
| 60:40 | $46^{c}\pm1.05$ | $89.63^{e}\pm0.13$ | $-0.20^{e}\pm0.01$ | $12.54^{g}\pm0.01$ |
| 50:50 | $50^{b}\pm1.30$ | $88.96^{f}\pm0.30$ | $-0.45^{f}\pm0.02$ | $14.10^{f}\pm0.02$ |
| 40:60 | $50^{b}\pm1.20$ | $88.48^{f}\pm0.30$ | $-0.53^{g}\pm0.01$ | $15.00^{e}\pm0.03$ |
| 30:70 | $50^{b}\pm1.52$ | $88.19^{f}\pm0.31$ | $-0.66^{h}\pm0.03$ | $15.96^{d}\pm0.01$ |
| 20:80 | $54^{a}\pm1.25$ | $87.66^{g}\pm0.12$ | $-0.75^{i}\pm0.05$ | $16.84^{c}\pm0.04$ |
| 10:90 | $54^{a}\pm1.10$ | $86.65^{h}\pm0.25$ | $-0.84^{j}\pm0.01$ | $18.29^{b}\pm0.02$ |
| 0:100 | $54^{a}\pm1.24$ | $86.25^{h}\pm0.30$ | $-0.90^{k}\pm0.01$ | $19.02^{a}\pm0.01$ |

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

process of bleaching and deodorization to remove color pigments and essentially all trace metals while cold-pressed RBO did not pass those processes (Ghosh, 2007; Chen *et al*., 2008; Hoed *et al*., 2010). By comparing the color of hydrogenated PKO and cold-pressed RBO to the Munsell Book of Color, it was found that level of color and brightness/ intensity of hydrogenated PKO was 5Y and 9/2, respectively, and for cold-pressed RBO it was 10R and 2.5/2, respectively. The lightness (L*) of pure hydrogenated PKO (93.06) was higher than that of pure cold-pressed RBO (86.25) ($P \leq 0.05$) since it has been processed by refining, bleaching and deodorization. However, the L* of cold-pressed RBO (86.25) in this present study was much higher than the values reported by Jennings and Akoh (2009) and Thanonkaew *et al*. (2012) which were 34.50 and 10.44, respectively. Due mainly to the dark brown color of cold-pressed RBO, as its ratio increased, the L* and redness (a*) significantly decreased while the yellowness (b*) increased ($P \leq 0.05$).

### 3.2 Profile of fatty acids

The 10 fatty acids (FA) presented in hydrogenated PKO in descending order were lauric, stearic, myristic, palmitic, capric, caprylic, elaidic, oleic, arachidic and caproic acids. In contrast, the 5 FA found in cold-pressed RBO in descending order were oleic, linoleic, palmitic, stearic and linolenic acids, respectively (Tables 2 and 3), similar to those reported by Singanusong *et al.* (2010). The saturated fatty acid (SFA) composition of hydrogenated PKO, cold-pressed RBO and their mixtures is shown in Table 2. Hydrogenated PKO consisted of medium-chain (6:0-12:0) and long-chain FA (14:0-20:0) but cold-pressed RBO had only long-chain FA (16:0-18:0). These findings are consistent with those of Akpanabiatu *et al.* (2001) and Kok *et al.* (2011) for kernel of oil palm and the CODEXSTANDARD 210-1999 (Codex Alimentarius Commission, 2011) for palm kernel oil and Singanusong and Noitup (2012) for cold-pressed RBO. Lauric acid was the predominant SFA in hydrogenated PKO (45.48 g/100 g), therefore, the amount was slightly decreased with decreasing ratio of hydrogenated PKO. On the other hand, palmitic acid was the major SFA found in cold-pressed RBO (20.00 g/100 g), which was consistent with the findings of Chotimarkorn and Silalai (2008) (20.70g/100 g) but higher than that reported by Singanusong and Noitup (2012) (13.71 g/100 g). The total SFA decreased with increasing ratio of cold-pressed RBO. In contrast, the total unsaturated fatty acid (UFA) increased with decreasing ratio of hydrogenated PKO (Table 3). The major UFA found in cold-pressed RBO were oleic and linoleic acids; 44.30 and 30.80 g/100 g, respectively. The data were in agreement with findings of Chotimarkorn and Silalai (2008) who stated that oleic and linoleic acid contents in cold-pressed RBO were 44.10 and 28.10 g/ 100 g, respectively. However, the values were higher than those reported by Singanusong and Noitup (2012), who stated that oleic and linoleic acid contents in cold-pressed RBO were 27.18 and 21.62 g/100 g, respectively. The differences could be due to differences in genetic and environmental factors, time of harvest and extraction methods.

Pure hydrogenated PKO contained mainly SFA, a few monounsaturated fatty acids (MUFA) and no polyunsaturated fatty acids (PUFA) whereas pure cold-pressed RBO contained mainly MUFA, following by PUFA and SFA (Table 4). The increase in the ratio of cold-pressed RBO would increase the levels of MUFA and PUFA and decrease the levels of SFA in the mixtures. This was also obviously evident by changing the Polyunsaturated : Monounsaturated : Saturated fatty acids (PMS) ratio as shown in Table 4 and Figure 1.

Table 2. Composition of saturated fatty acids of hydrogenated PKO, cold- pressed RBO and their mixtures.

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

Means in the column with different superscripts are significantly different ($P \leq 0.05$)

Table 3. Composition of unsaturated fatty acids of hydrogenated PKO, cold-pressed RBO and their mixtures.

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

Means in the column with different superscripts are significantly different ($P \leq 0.05$)

Table 4. Unsaturated and saturated fatty acid contents and PMS ratio of hydrogenated PKO, cold-pressed RBO and their mixtures.

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

Means in the column with different superscripts are significantly different ($P \leq 0.05$)
PMS ratio = Polyunsaturated : Monounsaturated : Saturated fatty acids ratio

### 3.3 Antioxidative compositions

As shown in Figure 2, γ-oryzanol was only found in cold-pressed RBO, therefore, any mixtures that contained cold-pressed RBO would have γ-oryzanol (Figures 2a). Pure cold-pressed RBO had significantly the highest γ-oryzanol content (1,155 mg/100 g oil) and antioxidant activity (94.24%) ($P \leq 0.05$) while pure hydrogenated PKO had no γ-oryzanol and significantly the lowest antioxidant activity (9.26%) ($P \leq 0.05$). The γ-oryzanol content of cold-pressed RBO was the same as the value reported by Singanusong and Noitup (2012) (1,155 mg/100 g oil) but higher than the reported values of Thanonkaew *et al*. (2012) (203.00 mg/100 g oil) and Chotimarkorn and Silalai (2008) (49.58 mg/100 g oil).

The α-tocopherol content increased with increasing ratio of cold-pressed RBO (Figure 2b); it was very low (0.09 mg/100 g oil) for hydrogenated PKO but much higher (30.82 mg/100g oil) for cold-pressed RBO. The α-tocopherol content of hydrogenated PKO was in the range of the values reported by the CODEXSTANDARD 210-1999 (Codex Alimentarius Commission, 2011) (Not Detected-4.4 mg/100 g oil), while the α-tocopherol content of cold-pressed RBO was

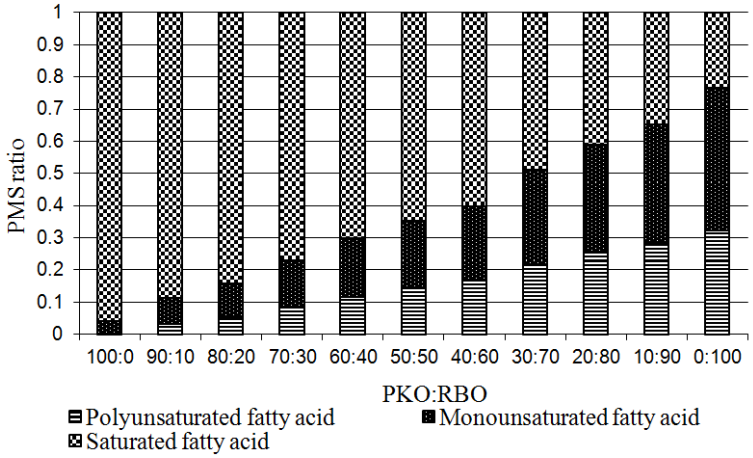


Figure 1. PMS ratio of fatty acid compositions in hydrogenated PKO, cold-pressed RBO and their mixtures.

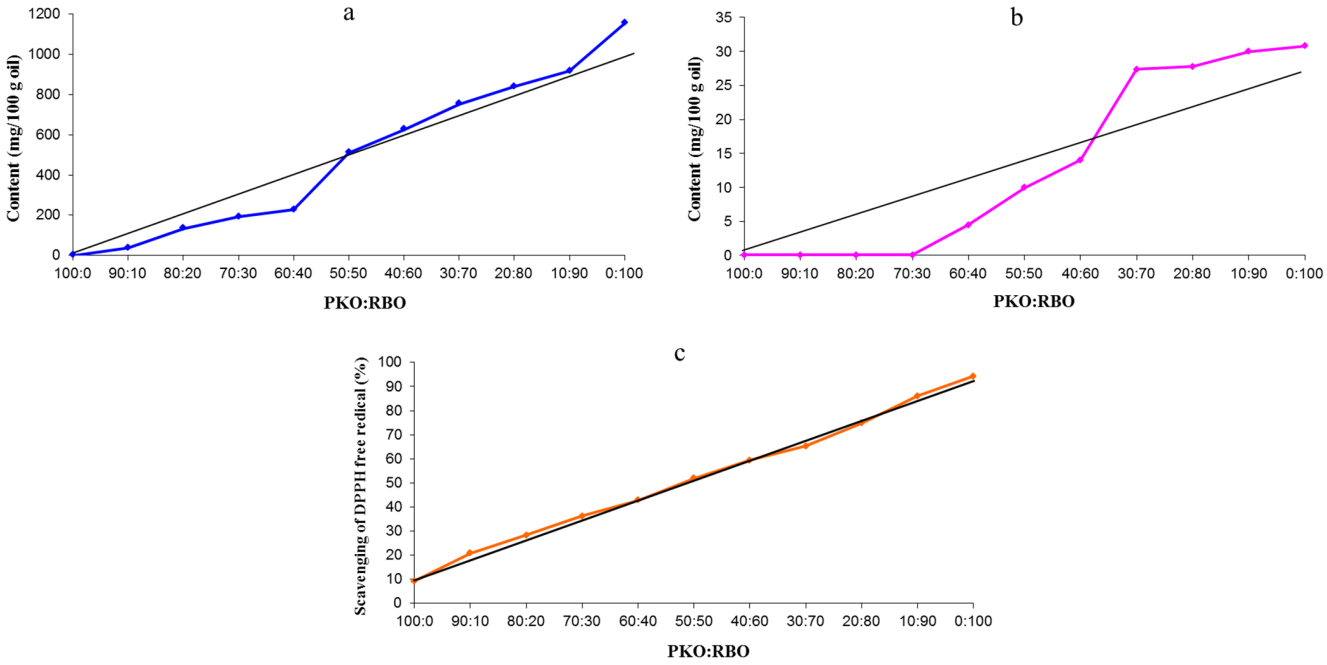


Figure 2. γ-Oryzanol content (a), α-tocopherol content (b) and antioxidant activity (c) of hydrogenated PKO, cold-pressed RBO and their mixtures.

higher than the reported value of Chotimarkorn and Silalai (2008) (6.91 mg/100 g oil) and the CODEXSTANDARD 210-1999 (Codex Alimentarius Commission, 2011) (4.9-5.83 mg/100 g oil).

Pure hydrogenated PKO had the lowest antioxidant activity while pure cold-pressed RBO had the highest ($P \leq 0.05$). The antioxidant activity increased significantly ($P \leq 0.05$) with increasing ratio of cold-pressed RBO (Figure 2c). It could be obviously concluded that the high antioxidant activity of cold-pressed RBO was mainly from γ-oryzanol as the trend lines of both γ-oryzanol (Figure 2a) and antioxidant activity (Figure 2c) closely fit to the result lines. The trend line of α-tocopherol did not well fit with the result line (Figure 2b). This might be due mainly to the fact that α-tocopherol content in the pure hydrogenated PKO and in the mixtures with 10-30% cold-pressed RBO was too low to be detected. Substitution of hydrogenated PKO by cold-pressed RBO from 40% would provide α-tocopherol and γ-oryzanol and hence antioxidant activity. Thanonkaew *et al.* (2012) found that cold-pressed RBO had antioxidant activity of 30% which was much lower than the value (94.24%) found in this present study. Genetic and environmental factors as well as extraction and analytical methods would contribute to these differences.

### 3.4 Trans-fat

Pure hydrogenated PKO had the highest trans-fat content ($P \leq 0.05$) while pure cold-pressed RBO had no trans-fat. Trans-fat was only detected in the hydrogenated PKO: cold-pressed RBO mixtures of 100:0, 90:10 and 80:20 (2.73, 1.93 and 1.85 g/100 g oil, respectively), indicating that substitution of hydrogenated PKO by cold-pressed RBO at 30% would reduce the level of trans-fat, hence the blended oils would offer reduced health risk from trans-fat as it has been reported to be a contributor to heart disease and cancer (Mensink and Kata, 1990; Ascherio *et al*., 1999; Hu *et al.*, 2001; Fernendez *et al*., 2007). A 2% increase in trans-fat intake has been calculated to increase the risk of coronary heart disease by 23% (Mozaffarian *et al*., 2006). The main sources of trans-fat are partially hydrogenated oils. The trans-fat consumption from refined edible oils is not particularly large as small amount of trans-fat are found in refined edible oils due to the high temperatures used during the deodorization step (Ceriani and Meirelles, 2007; Tsuzuki *et al*., 2010). Hou *et al.* (2012) investigated trans-fat in edible oil in China. They found that the total trans-fat of soybean, rapeseed, sunflower and corn oils were 1.15, 1.37, 1.41 and 2.01 g/100 g, respectively. These values were lower than the levels found in this present study, except for corn oil which was higher than that of PKO:cold-pressed RBO mixtures of 90:10 and 80:20. The difference could be due to types of oil used and the PKO used in this study has been hydrogenated which could contribute to trans-fat formation. Tsuzuki *et al.* (2010) studied the formation of trans-fat in 6 kinds of commercial edible vegetable oils during frying and heating process. They reported that the amount of total trans-fat in these fresh oils was in the range of 1.42-2.08 g/100 g and small changes in trans-fat were observed after 4 h heating. They also concluded that an ordinary frying process using unhydrogenated edible oils has little impact on total trans-fat intake from edible oils. Furthermore, Liu *et al.* (2007) analyzed trans-fat in unhydrogenated and hydrogenated soybean oil during heating. They found that no trans-fat formation was observed even after extensive heating of unhydrogenated and hydrogenated soybean oil at 160, 180 or 200°C for 24 h, implying that trans-fat can only be formed under drastic heating condition. These data suggested that differences in types of vegetable oil, refinement process, frying conditions and methods of trans-fat measurement would contribute to differences in trans-fat levels found in these studies.

## 4. Conclusions

This study illustrates that mixtures of hydrogenated PKO and cold-pressed RBO have various physical and chemical properties in terms of viscosity, color, trans-fat, fatty acid composition, γ-oryzanol, α-tocopherol and antioxidant activity. The hydrogenated PKO:cold-pressed RBO mixtures of 80-100:20-0 contained trans-fat, therefore, they should not be selected for further utilization in food products. Substitution of hydrogenated PKO by cold-pressed RBO from 30-100% would be suitable for food applications in terms of nutrition and health.

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