The Physical and Chemical Properties of Policosanol-Based Organogel Shortening for Replacing Saturated and Trans-Fat in Cookies

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ABSTRACT

Organogel is an edible oil gel formed by organogelator that are interesting to be used as alternative shortening with zero *trans*-fat and low saturated fat. Policosanol is a group of long-chain fatty alcohol that shows good organogelation property. It can be extracted from several sources such as sugar cane wax (SW), rice bran wax (RW), and beeswax (BW). The policosanol from different sources are varied in their compositions that may affect on the gelling ability. Therefore, the objective of this study was to investigate the fatty acid compositions, physical and chemical properties of policosanol-based organogel shortening for replacing saturated and *trans*-fat in cookies. Rice bran oil organogel shortening contained policosanol from SW, RW and BW were prepared. The results showed that all organogel shortenings had no *trans*-fat ($p<0.05$) with high content of monounsaturated fatty acid as in rice bran oil. Furthermore, the organogel shortening had lower saturated fat (23.77%) than that the commercial shortening (CS) (34.39%). The stability index and firmness value were ordered from CS, rice bran wax policosanol shortening (RWS), sugarcane wax policosanol shortening (SWS), and beeswax policosanol shortening (BWS), respectively. The lowest peroxide value was CS (1.32 meq/ kg oil) and followed by BWS (1.46 meq/ kg oil) whereas there was no significant difference in the acid value between the commercial and organogel shortening (0.03 ± 0.00 mg KOH/g oil). It was also found that the replacement of shortening with organogel produced cookies with high hardness and crispiness than that the CS cookies. Furthermore, all organogel shortening cookies had low saturated fat and zero *trans*-fat. Thus, policosanol-based organogel shortening can be used as alternative shortening in cookies.

Keywords: Organogel, Shortening, Policosanol, Cookies

INTRODUCTION

Shortening is a solid fat which plays an important role in bakery products such as cake, pastry and cookies (Mert & Demirkesen, 2016). It is a solid fat derived from liquid vegetable oil by modifying with different processes such as hydrogenation, partial-hydrogenation, fractionation and interesterification

Article history:
Received 17 September 2019; Received in revised form 16 October 2019;
Accepted 28 November 2019; Available online 2 June 2020

(Metzroth, 2005). However, by using these processes, the saturated fatty acids (SFA) are also increased and the *trans* fatty acid (TFA) may occur (Öğütcü et al., 2015a). Consumption of high SFA and TFA is associated with the risk of cardiovascular diseases (CVD) which is the one cause of death globally (Patel and Dewettinck 2016). Moreover, the recommendations about dietary fat suggest that the nutritional profile will be improved if the SFA is used instead of unsaturated fatty acid (Bier, 2016).

Vegetable oils are the source of unsaturated fatty acid. It is well known that consumption of unsaturated fatty acid is beneficial for health. Monounsaturated fatty acids (MUFA) have been reported to reduce LDL cholesterol whereas HDL cholesterol might be increased (Abedi & Sahari, 2014). The effects of polyunsaturated fatty acids (PUFA) have been shown the effect on human health in the prevention of cardiovascular disease, coronary heart disease, and cancer (Carluccio et al., 2007). In addition, most vegetable oils contain vitamins and phenolic compounds which are beneficial for health (Orsavova et al., 2015). The World Health Organization (WHO) recommended the ratio of SFA and MUFA and PUFA at 1:1.5:1 (WHO, 2010). Rice bran oil is one of vegetable oil that contains high content of unsaturated fatty acids with the ratio SFA to MUFA and PUFA of 22.5: 44: 33.5 (%) which is close to that recommended by WHO. In addition, it contains oryzanol-a special phytosterol that found only in rice bran oil (Choudhary, Grover & Kaur, 2015).

Recently, a new technique called "organogelation" has received attention as an alternative way to produce zero *trans* fat and low SFA fat-containing products (Co & Marangoni, 2012). This technique can structure the edible oils without changing their fatty acid composition (Lim, Hwang & Lee, 2017). Organogel is composed of edible oil and organogelator (gelling agent). Therefore, the physical properties of the organogels depend largely by the type and concentration of the organogelators (Yılmaz & Öğütcü, 2015b). There are many substances that can be used as organogelators such as lecithin, monoacylglycerols, a mixture of phytosterol and oryzanol, fatty alcohol, and plant waxes (Hwang et al., 2013). Among these, the policosanol showed ability to form a gel at low concentrations (Lupi et al., 2013). Policosanol is a group of long-chain fatty alcohols derived from natural waxy materials such as sugar cane wax, rice bran wax, beeswax and wheat germ (Cherif et al., 2010). The compositions of policosanol are varied depending on the waxy sources. For example, sugarcane wax has octacosanol as the main component, while beeswax and rice bran wax have triacontanol as the main component (Lilitchan & Aryusuk, 2008). Moreover, Xu et al., (2007) reported that policosanol has positively effect on human health to prevent cardiovascular disease. Nowadays, many countries use policosanol as a dietary supplement (Irmak, Dunford & Milligan, 2006). In addition, our preliminary study found that rice bran oil organogel emulsion can be prepared with policosanol.

This work aimed to extend the previous study by preparing rice bran oil organogel shortening using policosanol from SW, RW and BW as organogelator to limit the saturated and *trans* fatty acid in cookies. The fatty acid compositions, physical and chemical properties of the organogel shortening were determined and compared with commercial shortening. Then, the organogel shortening were applied in cookies and its fatty acid compositions and texture property were determined.

MATERAILS AND METHODS

Materials

Refine rice bran oil was purchased from local supermarket (Bangkok, Thailand). The policosanols of rice bran wax, sugar cane wax and beeswax were purchased from Xian Biof Bio-Technology.Co.,Ltd. (China). Palsgaard® 6111 was obtained from Palsgaard A/S, DPO (Thailand) Ltd.

Preparation of organogel shortening

Method for preparation of organogel shortening was adapted from Yilmaz & Ogutcu, (2015a). Briefly, four percent of policosanol from rice bran wax, sugar cane wax and beeswax and stabilizer (Palsgaard 6111) were dissolved in rice bran oil in a 250-ml beaker and warm at 85 °C. The 25 ± 0.1 g of samples were weighed and transferred into a plastic cup and cooled down in an ice bath. Then, the samples were kept in a freezer for 1 h. After that, the samples were stored refrigerated at 4-5 °C until tested.

The fatty acid compositions

Four organogel shortening samples were converted to fatty acid methyl ester (FAME) by basic catalyzed transesterification as described by Kaewkool et al., (2009). Briefly, 500 μL of oil samples in toluene (about 2 mg/mL each) were mixed with 0.5 mL methanol, and then transesterified by passing the mixture through a micro-reactor packed with NaOH powder. The micro-reactor washed with 1 mL of toluene-alcohol (1:1 v/v) mixture for 20 s. The elution sample and washed solvent were combined, adjusted with 0.1 ml acetic acid and washed with 1 ml water. The organic phase was separated and dried over anhydrous Na_2SO_4. Gas chromatography (GC) analysis was carried out on a Shimadzu gas chromatograph model 2010 equipped with a flame-ionization detector (Shimadzu Inc., Tokyo, Japan). The GC conditions were as follow: helium as carrier gas, split ratio of 80/1, detector and injector temperature of 250 ^{o}C and oven temperature of 180 ^{o}C. Identification of FAMEs was performed using the equivalent chain length (ECL) or equivalent carbon number (ECN) method proposed by Krisnangkura et al., (1997). The fatty acid contents were reported as percentage area of each peak (Krisnangkura et al., 1997).

Texture property of organogel shortening

The firmness values of the organogel shortening were measured with a Texture Analyzer TA-XT2i by using a 45° conic probe. The method of the penetration test is select with 3.0 mm/s penetration speed into 23 mm depth, and then the probe is pulled out from the sample at 10 mm/s speed (Sonwai & Luangsasipong, 2013).

Stability index

Organogel shortening was weighed 20 ± 0.1 g in a centrifuge tube. The sample was kept in freezer at -20˚C for 20 hrs. Then, the sample was melt at room temperature and centrifuged at 5,000 rpm for 15 minutes. The separated oil was weighted. The stability index was calculated as percentage (%) of oil release (Da Pieve et al., 2010) as follow.

Oil release (%) = [Mass of separated oil (g) / Total mass of samples (g)] x 100

Acid value

One gram of sample was added into 9 ml alcohol-ether mixture and shaken well. Then two drops of phenolphalene was added. The solution was titrated with 0.01 N potassium hydroxide (KOH) until the pink color was observed and the acid value was calculated (AOAC, 2000).

Oleic acid (%) = S×M×28.2/W

When

S = volume of KOH used for titration of the sample - KOH used for titration of blank (ml)

M = Concentration of KOH (N)

W = Weight of sample (g)

Acid value (mg KOH/ 1 g oil) = % Free fatty acid (as oleic acid) x 1.99

Peroxide value

Five gram of sample was dissolved in 30 ml acetic acid: chloroform (3:2) in an Erlenmeyer flask. Then, 0.5 ml of potassium iodide was added. The solution was shaken for a minute and titrated with 0.01 N sodium thiosulfate by using starch solution as indicator. The peroxide value was calculated according to AOAC, (2000).

Peroxide value (meq/kg oil) = S ×M × 1,000 / W

When

S = Volume of $Na_2S_2O_3$ used in the sample titration - volume of $Na_2S_2O_3$ used in the blank titration (ml)

M = Concentration of $Na_2S_2O_3$ (N)

W = Weight of sample (g)

Preparation of cookies

The cookies were prepared by the method modified from AACC, (2000). The cookie dough recipe based on 100 g of flour contained 25 g sugar, 50 g shortening, 0.5 g salt, 1 g sodium bicarbonate, 0.5 g ammonium bicarbonate, 1.5 g of syrup and 9 g skim milk. Firstly, the sugar, salt, ammonium bicarbonate and sodium bicarbonate was mixed by using a mixer for 3 min at 85 rpm. Then, the organogel shortening or commercial shortening was added and mixed for additional 2 min at 85 rpm and 1 min at 140 rpm. In a separate container, the syrup and skim milk were mixed and then the mixture was added into the forming mixture and mixed again at 85 rpm for 2 min. Finally, flour was added and mixed for 4 min at 85 rpm and for 2 min at 150 rpm and kneaded by hand for 2 minutes. The kneaded dough was divided into two equal portions and then the dough pieces were sheeted to a thickness of 5 mm by using a pilot scale dough sheeter and cut using a 50 mm diameter circular die. Finally, cookie dough was baked in a conventional oven at 180 °C for 11 min.

Texture properties of cookies

The hardness and crispiness values of the cookies were determined according to the technique modified by Piga et al., (2005). A 2 mm cylindrical probe with a 1 kg load cell was used for the puncture test. Firstly, samples of cookies were placed in the centre and fixed on the heavy duty platform. The puncture test was performed with gradients of 3.0 mm/s entrance, 0.5 mm/s inside sample, and 10 mm/s backing speed until 7 mm deep with 20 g triggering force.

The oil extraction from cookies

The cookies were grinded. One hundred gram of the grinded cookies was mixed with petroleum ether 100 ml using shaker at room temperature to extract the oil. Then, the sample was filtrated to separate the oil fraction. The petroleum ether was removed by rotary evaporator. The fatty acid composition of the oil samples were determined (Sung & Lin, 2017).

Statistical analysis

The experimental were performed in triplicate. The results reported as an average value ± standard deviation and statistically analyzed by one-way ANOVA using SPSS program version 18 at significant differences ($P < 0.05$). The means were compared by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Shortening determination

The color and appearance of the prepared and commercial shortenings (CS) can be visualized in Fig. 1. It showed that the color of CS was white which was different from organogel shortening which showed in light yellow. This may be due to crystallization characteristic of the policosanol in the organogel shortening and that should be studied further. However, the appearances of all organogel shortenings were acceptable to be defined as solid fat.

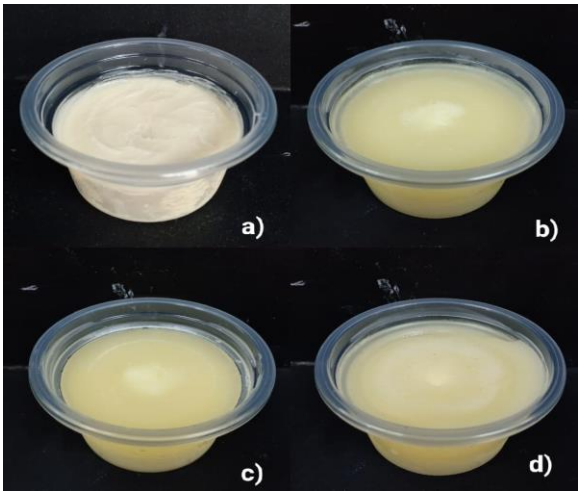

Figure 1 a) CS: Commercial shortening b) BWS: Organogel shortening with beeswax policosanol c) RWS: Organogel shortening with rice bran wax policosanol d) SWS: Organogel shortening with sugar cane wax policosanol

The fatty acid composition of shortenings

Table 1 The fatty acid composition of rice bran oil (RBO) and shortening

Fatty acids (%)	RBO	CS	BWS	RWS	SWS
C14:0	0.40 ± 0.01	0.46 ± 0.03	0.38 ± 0.01	0.33 ± 0.03	0.36 ± 0.01
C16:0	20.16 ± 0.25	27.15 ± 1.38	19.96 ± 0.02	19.92 ± 0.01	19.79 ± 0.17
C18:0	2.03 ± 0.05	3.01 ± 0.30	1.96 ± 0.01	2.13 ± 0.01	1.97 ± 0.00
C18:1	41.91 ± 0.32	36.95 ± 0.98	41.55 ± 0.23	42.35 ± 0.07	41.63 ± 0.20
C18:2	32.37 ± 0.46	29.29 ± 1.07	33.24 ± 0.16	32.15 ± 0.01	33.27 ± 0.14
C18:3	0.90 ± 0.02	0.92 ± 0.04	0.98 ± 0.04	0.97 ± 0.01	0.98 ± 0.02
C20:0	0.84 ± 0.04	1.09 ± 0.09	0.80 ± 0.02	0.81 ± 0.02	0.82 ± 0.02
C20:1	0.54 ± 0.02	0.42 ± 0.03	0.49 ± 0.04	0.47 ± 0.01	0.49 ± 0.04
C22:0	0.26 ± 0.01	nd	0.28 ± 0.02	0.50 ± 0.01	0.29 ± 0.01
C24:0	0.38 ± 0.01	0.58 ± 0.08	0.37 ± 0.03	0.37 ± 0.01	0.39 ± 0.01

Fatty acids (%)	RBO	CS	BWS	RWS	SWS
SFA	24.07 ± 0.18[a]	32.29 ± 1.82[b]	23.74 ± 0.15[a]	24.06 ± 0.04[a]	23.63 ± 0.12[a]
MUFA	42.65 ± 0.29[b]	37.38 ± 0.99[a]	42.04 ± 0.26[b]	42.82 ± 0.06[b]	42.12 ± 0.24[b]
PUFA	33.28 ± 0.47[b]	30.34 ± 0.84[a]	34.22 ± 0.12[c]	33.12 ± 0.02[b]	34.25 ± 0.13[c]
TFA	nd	nd	nd	nd	nd

Data are expressed as percentage of fatty acid; nd means not detected. The different letters in same row indicate significantly different ($p<0.05$), n=3.

Table 1 show the fatty acid composition of commercial- and the prepared-rice bran oil shortening using of different policosanol as organogelator. All shortening contained no TFA. The commercial shortening (CS) composed the most SFA (32.39%). On the other hand, shortening prepared by using policosanol from beeswax (BWS), rice bran wax (RWS) and sugarcane wax (SWS) as organogelator were contained only 23.63-24.18% SFA and with a high content of MUFA (42.04-43.06%). The results showed that both SFA and MUFA of all organogel shortenings were not with significantly different from the rice bran oil ($p<0.05$). In other word, the fatty acid compositions of organogel shortening were similar to rice bran oil. Therefore, it may be concluded that the fatty acid composition of organogel shortening from rice bran oil has not been altered by the organogelation process. Thus, these organogel shortening can be used as plastic fat for bakery products with nutritional advantages.

The physical properties of shortenings

Stability index

Stability index is measured by % oil release, which this low value is highly stable. The results are shown in Table 2. The stability index (% oil release) of CS, BWS, RWS and SWS were significantly different ($p<0.05$). The most stable shortening was CS. The stability of organogel shortening was in the order as follows: RWS > SWS > BWS. It is interesting to be noted that the stability of organogel shortening were different among BWS, RWS, and SWS. It may imply that various sources of policosanol had an effect on the stability of organogel shortening. Thus, compositions of policosanol of different sources were investigated. Rice bran wax and sugar cane wax contain long-chain hydrocarbon but beeswax contains short-chain hydrocarbons (Lan, 2019). The chain length of hydrocarbons can act on the gelling ability. Likewise, Hwang et al., (2012) evaluated the gelling ability of waxes and found that wax with longer chain showed better gelling ability than the shorter chain.

Firmness value

The texture of the shortening was measured in term of firmness value and shows in Table 2. The firmness value of CS was higher than the organogel shortening ($p<0.05$) due to it contained high content of SFA. That was in accordance with other studies. Ogutcu & Yilmaz, (2015a) found that the firmness value of the commercial shortening was higher than organogel shortening. However, they argued that the firmness of organogel was still high enough for the organogel to behave as solid fat. From Table 2 the organogel shortening, BWS had the lowest firmness and the RWS had the highest firmness. The results also showed that the firmness value was consistent with the stability index.

Table 2 The stability index and firmness value of shortening

Samples	Stability index (% oil release)	Firmness value (N)
CS	0.00 ± 0.00^{a}	4.21 ± 0.63^{c}
BWS	7.68 ± 0.22^{d}	1.53 ± 0.07^{a}
RWS	4.59 ± 0.35^{b}	2.78 ± 0.17^{b}
SWS	6.25 ± 0.22^{c}	1.92 ± 0.40^{a}

Value expressed as means ± standard deviations. The different letters in same column indicate significantly different ($p<0.05$), n=5.

The chemical properties of shortening

Table 3 The acid value and peroxide value of shortening

Samples	Acid value (mg KOH/ oil 1 g)	Peroxide value (meq/ kg oil)
CS	0.03 ± 0.00^{a}	1.32 ± 0.06^{a}
BWS	0.03 ± 0.00^{a}	1.46 ± 0.06^{b}
RWS	0.03 ± 0.00^{a}	1.70 ± 0.10^{c}
SWS	0.03 ± 0.00^{a}	1.76 ± 0.06^{c}

Value expressed as means ± standard deviations. The different letters in same column indicate significantly different ($p<0.05$), n=3.

Acid value and peroxide value

Acid values and peroxide values of the shortening are shown in Table 3. The acid values of all samples were 0.03 mg KOH/1 g oil. The CS had the lowest peroxide value and had significantly different ($p<0.05$) from the values of organogel shortening. The result was in accordance to the stability index which the CS was the most stable shortening. BWS had the lowest peroxide value among RWS and SWS. This is according to the results of Hwang et al., (2018) that they found the organogel with beeswax had lower PV than the organogel with rice bran wax. However, the

peroxide value of all shortening was showed low value. Therefore, it is believed that the organogel shortening was withstanding the oxidation.

Cookies determination

Figure 2 shows the appearance of the cookies samples namely a) cookies from commercial shortening (CS-C) b) cookies from organogel shortening with beeswax-policosanol (BWS-C) c) cookies from organogel shortening with rice bran wax-policosanol (RWS-C) and d) cookies from organogel shortening with sugar cane wax-policosanol (SWS-C). It showed that the color of all organogel shortening cookies were darker than the CS-C. In addition, the organogel shortenings increased hardness and crispiness of the cookies.

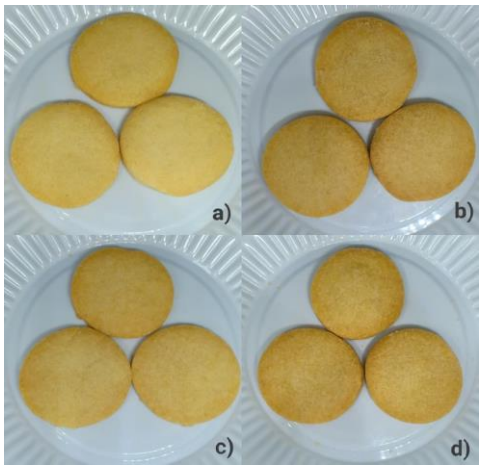

Figure 2 Visual appearance of cookies prepared with a) commercial shortening (CS-C), b) organogel shortening from beewax-policosanol (BWS-C), c) organogel shortening from rice bran wax-policosanol (RWS-C) d) organogel shortening from sugar cane wax-policosanol (SWS-C)

The fatty acid composition of the oil extracted from cookies

Table 4 The fatty acid composition of the oil extracted from cookies

Fatty acids (%)	CS-C	BWS-C	RWS-C	SWS-C
C14:0	0.44 ± 0.02	0.38 ± 0.01	0.39 ± 0.01	0.39 ± 0.00
C16:0	26.11± 0.18	19.69 ± 0.02	20.06 ± 0.06	20.19 ± 0.42
C16:1	nd	nd	0.18 ± 0.01	0.17 ± 0.02
C18:0	2.83 ± 0.04	2.17 ± 0.01	2.08 ± 0.08	2.39 ± 0.01
C18:1	37.24 ± 0.01	41.00 ± 0.06	41.05 ± 0.03	40.31 ± 0.62
C18:2	29.61 ± 0.22	33.60 ± 0.17	33.19 ± 0.18	33.26 ± 0.60
C18:3	0.3± 0.05	1.04 ± 0.02	1.01 ± 0.01	0.99 ± 0.08
C20:0	1.05 ± 0.04	0.86 ± 0.04	0.82 ± 0.04	0.88 ± 0.06
C20:1	0.43 ± 0.03	0.48 ± 0.01	0.47 ± 0.00	0.39 ± 0.15

Fatty acids (%)	CS-C	BWS-C	RWS-C	SWS-C
C20:3	0.32 ± 0.02	0.36 ± 0.02	0.33 ± 0.02	0.62 ± 0.06
C20:5	0.47 ± 0.12	nd	nd	nd
C24:0	0.57±0.08	0.43±0.02	0.40±0.06	0.41 ±0.05
SFA	$31.01±0.01^{c}$	$23.53±0.07^{a}$	$23.75±0.11^{a}$	$24.26±0.33^{b}$
MUFA	$37.67±0.02^{a}$	$41.47±0.07^{bc}$	$41.70±0.01^{c}$	$40.87±0.78^{b}$
PUFA	$31.32±0.03^{a}$	$35.00±0.13^{b}$	$34.55±0.13^{b}$	$34.87±0.46^{b}$
TFA	nd	nd	nd	nd

Data are expressed as percentage of fatty acid; nd means not detected. The different letters in same row indicate significantly different ($p<0.05$), n=3.

Table 4 shows fatty acid compositions of the oil extracted from the cookies. The results showed that fatty acid compositions of the extracted oil from cookies and the parent oil in shortening were very similar (Table 1 and Table 4). The CS-C had the highest SFA content compared to the organogel shortening cookies. BWS-C, RWS-C and SWS-C had low SFA and high content of MUFA. In addition, the TFA was not found in any cookies samples ($p <0.05$).

Table 5 Hardness and crispness value of cookies

Samples	Hardness (N)	Crispness
CS-C	$8.92 ± 0.13^{a}$	$21.50 ± 0.24^{a}$
BWS-C	$10.21 ± 0.16^{b}$	$25.55 ± 0.35^{b}$
RWS-C	$10.40 ± 0.10^{c}$	$29.09 ± 0.28^{d}$
SWS-C	$10.54 ± 0.10^{c}$	$27.42 ± 0.36^{c}$

Value expressed as means ± standard deviations. The different letters in same column indicate significantly different ($p<0.05$), n=5.

Hardness and crispness value of all cookies are shown in Table 5. The hardness and crispness of CS-C were lower than of the other cookies. Since CS-C contained more solid-fat than that the organogel shortening cookies and thus affected on the hardness and crispness of the cookies (Jacob & Leelavathi, 2007). The study of Sung & Lin, (2017) revealed that cookies made with commercial shortening had lower hardness than the cookies made with organogel while the cookies made with liquid oil had the highest hardness. Abboud et al., (1985) reported that the hardness was attributed to lacking proper aeration. The experiment results showed that the policosanol promote the formation of the three-dimensional network of the solid phase in the cookie matrix, prevent separating out of the liquid oil. This result could be also attributed to the high hardness and crispness of the organogel shortening cookies which play a positive role in prevent liquid oil leak out during and after baking.

CONCLUSIONS

Policosanol-based organogel shortening had characteristic like semi-solid. They can be used as shortening in bakery products. They contained 23.63-24.18% SFA, 42.04-43.06% MUFA and 33.15-34.25% PUFA. Although, the stability index and firmness value were slightly lower than that of the commercial shortening, they were acceptable to be defined as solid fat. However, the stability index and firmness value can be improved in the further study. Moreover, organogel shortening had lower saturated fatty acids than commercial shortening. Furthermore, policosanol-based organogel shortening can apply in cookies. It showed that organogel shortening cookies had higher hardness and crispness value than commercial shortening cookies. Moreover, organogel shortening cookies were in low SFA and zero *trans*-fat. Thus, policosanol-based organogel shortening can be used as alternative shortening in cookies.

ACKNOWLEDGMENTS

This work was supported by the Agricultural Research Development Agency (Public Organization), contract No. CRP6205030850. The authors would like to thank Medifoods (Thailand) Co., Ltd. for donating rice bran oil and Palsgaard A/S, DPO (Thailand) Ltd. for donating stabilizer (Palsgaard® 6111).

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