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Effects of Surfactants and Aging Time on Solidification of Rice Bran Oil at Room Temperature

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Abstract: This study investigated the roles of rice mono- and diacylglycerol (rice MDG) and commercial MDG on solid structure formation of rice bran oil (RBO) and RBO-anhydrous milk fat (AMF) blends after the crystallized blends were aged at 5℃ for 12 or 24 h and stored at 30℃ for 12 or 24 h. The rice MDG was prepared using a pilot-scale molecular distillation (MD) unit to evaporate out the free fatty acids from deodorizer distillate (DD) at 120, 140 and 160℃ at 0.1 Pa. It was found that increasing the distillation temperature during MD process from 120℃ to 140℃ resulted in higher contents of rice MDG and γ-oryzanol in the unevaporated fraction (UMD) compared to those in DD. Although UMD increased solid fat content in RBO-UMD blend, it could not stabilize the solid fat phase in the RBO-UMD or RBO-AMF-UMD oleogel at 30℃ storage. In the presence of UMD, RBO-AMF-UMD blends remained in a liquid state although it contained a high content (38.54%) of saturated fatty acids. On the other hand, with the addition of commercial MDG rich in palmitic acid, RBO-MDG and RBO-AMF-MDG blends were able to retain the volume of solid fat phase in the oleogels provided that the RBO-MDG and RBO-AMF-MDG oleogels were aged at 5℃ for at least 12 h. This study implicated that the presence of 1% MDG surfactant having different acyl chains from the major fatty acids in the bulk oil phase, as well as aging regime, could be used to assist solid structure forming process of RBO and RBO-AMF oleogels.

Key words: aging, deodorizer, oleogel, rice, surfactant

1 INTRODUCTION

Fats have been used in food formulation as they provide functionalities such as hardness, viscosity, plasticity and spreadability in fat-containing food products[1]. The main component in fat is triacylglycerols (TAGs), which is responsible for fat crystallization and the formation of fat crystal network required in the textural characteristics of fat and food products. Different arrangement of TAG molecules, both in liquid phase and crystalline phase, results in different thermo-physical characteristics of fat functionalities[2, 3].

The viscoelastic properties related to mouthfeel and hardness from fat crystal network of *trans* and saturated fat are required in food products. However, consuming saturated fat and *trans* fat diets could lead to metabolic syndromes and cardiovascular diseases due to the increasing of low density lipoprotein cholesterol and TAGs in blood. Recently, attempts have been made of modify fat solid structure that could provide similar functionalities to those from saturated and *trans* fat crystalline structure. Mechanisms involved in stabilizing such three-dimensional network in solid structure include traditional fat crystal network of TAGs and the non-traditional structuring network of organogelators, which resulted in the formation of oleogels.

In traditional fat crystal network, such as butter, the liquid fraction was entrapped in crystalline fat network, which was around 12% solid fat content (SFC) at 25℃[4]. After nucleation and the formation of lamella TAGs, the lamella would stack to each other in the ordered fashion and form crystallite, which would assemble into flocs and final macroscopic network[5]. The solid fat existed in a three-dimensional fat crystal network is responsible for fat functionalities *via* the size of crystal, the types of crystal polymorphs, the strength of the links between the crystal, the geometry of the network, and the network density. Therefore, the composition of fatty acids in TAGs and fat processing conditions mainly govern the characteristics of

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fat and fat-containing food products[5].

The oleogel, however, is a bi-continuous colloidal system containing liquid organic phase such as edible oil and the gelling agent called organogelator. The organogelators have the characteristics of self-assembling or being a polymeric strand. Thus, they can form continuous gel network entrapping liquid oil in their structure[6, 7], resulting in solidified oil or oleogel. Continuous gel network having numerous structures could be categorized according to the types of organogelator as self-assembled crystalline surfactants[8]; self-assembled fibril of phytosteryl moieties[9]; and the carbohydrate polymers[6].

The most common non-ionic surfactant widely used in the food industry is monoacylglycerol (MAG)[8, 10]. It is commercially produced by direct esterification of glycerol and fatty acids, or by interesterification of TAGs from fat or hydrogenated fat with glycerol. The surfactants from both processes usually contain 35–50% MAG, 46–48% diacylglycerol (DAG), and the rest is TAG. Commercial MAG is usually in the form of solid and has a high melting temperature, i.e. 80℃[11]. Although DAG has limited use in the food industry compared with MAG, it is important in the cosmeceutical industry due to its low melting temperature[12].

When non-ionic surfactants having low hydrophile-lipophile-balance (HLB) such as MAG are present in liquid oil phase, the elastic oleogel network could be formed, particularly with appropriate aging temperature and time[13, 14]. This is because the reverse bilayer of MAG in oil can spontaneously form. The lateral area of reverse bilayer is the hydrophobic part of aliphatic chain dipping into the continuous oil phase. Upon decreasing temperature to below the gelation temperature of MAG, the inverse bilayer of self-assembled MAG could subsequently grow and organize into inverse lamellar phase and sub-α crystal structure, entrapping the oil within its oleogel structure *via* capillary force[8, 13, 14]. It is apparent that aging process of liquid oil or solid fat containing non-ionic surfactant at low temperature could further modify fat functionalities in addition to the crystalline network of TAGs[13].

Deodorizer distillate (DD) from refining process of rice bran oil (RBO) is composed of FFA, MAG, DAG and bioactive compounds such as phytosterols, squalene and tocols[15–17]. It was found that molecular distillation (MD) process could be used to concentrate rice phytochemicals (such as phytosterols and γ-oryzanol) in the DD obtained from physical refining process[15]. It was hypothesized in the current study that under appropriate control of evaporating temperature and pressure, the components having boiling points higher than the FFAs would be retained in the unevaporated fractions (a so-called UMD), particularly rice phytochemicals, MAG and DAG, as crude mixture. To our knowledge, the use of UMD fractions obtained from physical refining process of RBO in controlling lipid/fat functionalities has never been reported.

This study therefore investigated further the influences of evaporating temperature during MD process on the chemical and physical characteristics of the UMDs after FFAs were evaporated out. The effects of rice mono- and diacylglycerol (rice MDG) in UMD fraction obtained from MD process, as well as the commercial MDG, on the formation of solidified RBO and RBO-anhydrous milk fat (AMF) blends were investigated under different aging regimes. The insights into the influence of surfactants and aging process on promoting or inhibiting fat crystallization or solidification of bulk liquid oil may help better understanding in structure-forming process of *trans*-free solidified oils.

2 EXPERIMENTAL

2.1 Materials

Physically refined rice bran oil (RBO) and the unevaporated fraction (UMD) obtained after distillation by a pilot-scale molecular distillation system were provided by Surin Bran Oil Co. (Surin, Thailand). Anhydrous milk fat (AMF) was purchased from Vicchi Enterprise Co. (Bangkok, Thailand). The fatty acids in RBO and AMF from one batch used in this study were summarized in Table 1[18].

Commercial mono- and diacylglycerol (MDG) was composed of more than 90% saturated fatty acids, most of which was palmitic acid *ca.* 62.4%. The commercial MDG was provided by Berli Jucker Public Co. (Bangkok, Thailand).

2.2 Characteristics of rice MDG concentrated by a pilot-scale molecular distillation unit

Deodorizer distillate (DD), a co-product from the de-acid step of the physical refining process of RBO, was used as the raw material for molecular distillation (MD) to evaporate FFAs. The unevaporated fractions, designated as UMDs, were obtained after processing at different operating temperatures of 120, 140 and 160℃ at 0.1 Pa. The UMDs were characterized as detailed below.

2.2.1 Thermal properties

Ten mg samples of UMDs obtained at different operating temperatures were weighed into individual stainless steel pans and analyzed by differential scanning calorimetry (DSC) (Pyris 1; PerkinElmer, Norwalk, CT, USA). The DSC program was set for the following cycle: heating from 25℃ to 90℃ at 10℃/min and holding at 90℃ for 3 min, cooling from 90℃ to −60℃ at 10℃/min and holding at −60℃ for 3 min, and heating from −60℃ to 90℃ at 10℃/min. The onset temperature (*To*) and end temperature of melting (*Te*) were determined.

2.2.2 Mono- and diacylglycerol ratio

The saponifiable matter in the UMDs obtained after molecular distillation at different temperatures was determined by high-performance thin-layer chromatography (HPTLC) (Camag, Berlin, Germany). First, a silica plate

Table 1 Fatty acid composition of physically refined rice bran oil (RBO), anhydrous milk fat (AMF) and rice bran oil blended with anhydrous milk fat (RBO-AMF) at a mass ratio of RBO to AMF of 0.75: 0.25.

Fatty acid	g/100 g RBO	g/100 g AMF	g/100g RBO-AMF blend (calculated)
Caprylic acid (C8:0)	–	1.36	0.34
Capric acid (C10:0)	–	5.20	1.30
Lauric acid (C12:0)	–	7.24	1.81
Myristic acid (C14:0)	0.51	17.58	4.78
Palmitic acid (C16:0)	24.21	33.22	20.46
Stearic acid (C18:0)	1.70	9.09	3.55
Arachidic acid (C20:0)	0.40	–	0.30
Total of saturated fatty acid	26.82	73.69	32.54
Palmitoleic acid (C16:1)	0.21	1.47	0.53
Oleic acid (C18:1, cis-9)	40.87	20.33	35.74
Linoleic acid (C18:2, cis)	29.07	0.85	22.02
γ-Linolenic acid (C18:3n6)	0.45	0.55	0.48
Eicosenoic acid(C20:1)	0.50	0.97	0.62
Total of unsaturated fatty acid	71.1	24.17	59.39

was pre-developed in hexane and diethyl ether using a ratio of 1:1 (v/v). The plate was activated at 110℃ to remove impurities. Next, 5, 10 and 15 μL of standards and the UMD samples were spotted near the bottom of the plate using a glass micro-syringe. The plate was first developed at a distance of 4.5 cm from the origin. The solvent system consisted of a mixture of methyl acetate: isopropanol: chloroform: methanol: 0.25% (w/v) KCl in a ratio of 25: 25: 25: 10: 9 by volume. The plate was dried over NaOH in a desiccator for 30 min. Second development of the plate was performed at a distance of 9.5 cm in a mixture of hexane: diethyl ether: glacial acetic acid in a ratio of 80: 20: 2 (v/v). Separate lipid classes were detected by spraying with 3% (w/v) cupric acetate in 8% (w/v) phosphoric acid, followed by charring at 160℃ for 20 min to visualize the bands[19].

2.2.3 γ-Oryzanol

The γ-oryzanol content in UMD samples was evaluated by spectrophotometric technique[20]. Briefly, 10 mg of sample was weighed into a 10 mL volumetric flask. Hexane was used to dissolve the sample and adjust the volume. The γ-oryzanol content was determined by measuring the absorbance at 314 nm using a UV-Vis spectrophotometer (Spectronic GENESYS 10; Thermo Scientific, Waltham, MA, USA). The γ-oryzanol content was then calculated as follows:

$$\gamma - oryzanol \text{ content (mg /100 } g) = \frac{Absorbance \text{ at 314 nm in hexane solution *10000}}{g \text{ of sample * 358.9}} \quad (1)$$

2.2.4 Thermal characteristics of rice bran oil (RBO) supplemented with the unevaporated fraction (UMD) and commercial mono-, diacylglycerol (MDG)

One g samples of RBO, RBO supplemented with 1% UMD obtained after distillation at 140℃, RBO supplemented with 1% commercial MDG, and RBO supplemented with 1% UMD and 1% commercial MDG were heated at 70℃. Fifteen mg samples were weighed into stainless steel pans and analyzed by DSC. The DSC program was set for the following cycle: heating from 25℃ to 60℃ at 10℃/min and holding at 60℃ for 3 min, cooling from 60℃ to −60℃ at 10℃/min and holding at −60℃ for 3 min, and heating from −60℃ to 60℃ at 10℃/min. The onset temperature (*To*) and end temperature of melting (*Te*) were determined. Solid fat content (SFC) was calculated by dividing the partial area under the melting curve by the total area from −60 to 60℃ and multiplying by 100[21].

2.2.5 Apparent viscosity of rice bran oil (RBO) mixed with the unevaporated fraction (UMD) or commercial mono-, diacylglycerol (MDG) before aging

RBO and RBO-AMF – in the absence or presence of 1% UMD obtained after distillation at 140℃, or 1% commercial MDG – were melted at 70℃ and cooled down to 25℃. The apparent viscosity of each sample was measured using a Brookfield viscometer equipped with a UL adapter (DV-III programmable rheometer; Brookfield Engineering, Middleborough, MA, USA) at 60 rpm and 25℃.

2.3 Effect of aging regime on solidification of RBO and RBO-AMF with added surfactants

RBO and RBO-AMF – in the absence or presence of 1% UMD obtained after distillation at 140℃, or 1% commercial MDG – were characterized for the volume of solid fat phase after aging at 5℃ and storing at 30℃. First, 1.5 L of samples with designated surfactants added were heated at 70℃ and then cooled to −22℃ in a scraped-surface freezer (KATOMO, China) at a cooling rate of 5.5℃/min to crystallize fat. Ten mL of each sample was put into a plastic 15 mL centrifuge tube with a marked volume. The samples were aged at 5℃ for 12 h or 24 h; then stored at 30℃ for 12 h or 24 h and the liquid oil was discarded. The opaque solid fat phase in the centrifuge tube was measured and calculated as % volume of solid fat phase.

2.4 Statistical analysis

Two different batches of samples were prepared for analyses. The data were analyzed by analysis of variance (ANOVA) at a significance level of $p<0.05$. All statistical analyses were performed using SPSS software version 12 (SPSS, Chicago, IL, USA).

3 RESULTS AND DISCUSSION

3.1 Chemical and physical characteristics of the unevaporated fractions (UMDs) obtained after molecular distillation

Figure 1 illustrates the thermograms of UMDs obtained after molecular distillation (MD) process at different temperatures, i.e. 120, 140 and 160℃, at 0.1 Pa. The UMDs obtained at distillation temperatures of 140 and 160℃ started melting at (-) 46℃. However, the UMD obtained at a distillation temperature of 120℃ started melting at (-) 29℃. All UMDs completely melted at 38℃. Similar thermograms of UMDs obtained after distillation at 140 and 160℃ suggested that both UMDs had similar constituents, but they were different from those of the UMD obtained after MD process at 120℃. Unlike the UMDs obtained from distillation at high temperature, the UMD obtained after distillation at 120℃ showed an endothermic peak between around (-) 29 and (-) 9℃.

The endothermic peak was most likely residual FFAs present in the UMD; this was confirmed by HPTLC chromatograms as a band of oleic acid, which was found only in the UMD obtained after distillation at 120℃ (Fig. 2). The constituents from UMDs obtained at different distillation temperature contained TAGs the most, followed by DAGs or MAGs depending on distillation temperature. The HPTLC chromatograms showed only triolein, diolein and monoolein as compared to the standards. Densitometric analysis revealed differences in the ratios of non-polar fractions of FFA: MAG: DAG: TAG in UMDs obtained after

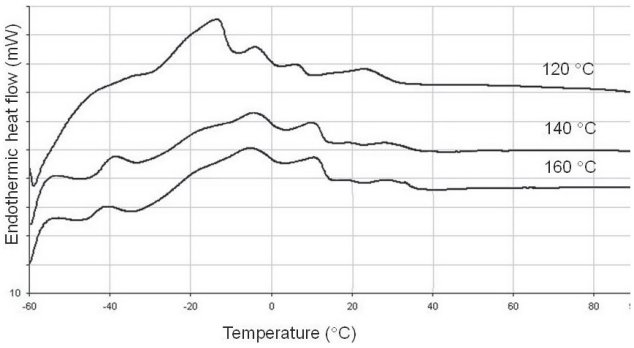

Fig. 1 Effect of distillation temperature during molecular distillation on melting profiles of the unevaporated fraction (UMD).

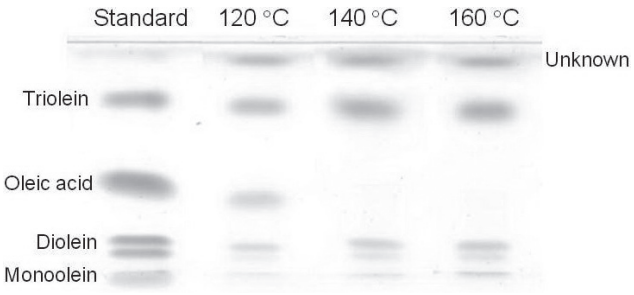

Fig. 2 Effect of distillation temperature during molecular distillation on the profile of non-polar compounds of the unevaporated fractions (UMD), determined by high-performance thin-layer chromatography.

distillation at different temperatures ($p<0.05$; Table 2). The increase in the distillation temperature from 120℃ to 140℃ markedly reduced FFA content. TAGs still remained at a level of more than 50% in all UMDs, regardless of distillation temperature. The increase in temperature from 140℃ to 160℃ during MD process, however, did not significantly change the γ-oryzanol content. The UMD obtained after distillation at 140℃ (UMD_{140}) was then used in further investigations as the source of rice UMD on physical characteristics of RBO blends, since the UMD_{140} contained no FFA, had high γ-oryzanol content, MAG and DAG (Table 2).

3.2 Effect of surfactants on thermal properties and apparent viscosity of RBO and RBO-AMF blends

The commercial MDG contained 62.39% palmitic acid according to manufacturer datasheet. It is apparent that the commercial MDG had high content of saturated fatty acid; while the majority of the MAG and DAG in UMD was monounsaturated fatty acid of oleyl esters. Thermograms of RBO containing UMD and/or commercial MDG are shown in Fig. 3. The addition of UMD and/or commercial MDG did not significantly change the melting characteristics of RBO although they were different in terms of chemi-

Table 2 Effect of distillation temperature during molecular distillation on the mass ratios of non-polar fractions and γ-oryzanol content in the unevaporated fraction (UMD).

Characteristics	Distillation temperature during molecular distillation		
	120℃	140℃	160℃
Melting temperature range (℃)			
First range	Not detected	(-)46.2 - (-)32.9	(-)46.2 - (-)32.9
Second range	(-)29.1 - (-)8.5	Not detected	Not detected
Third range	(-)10.2 - 37.8	(-)10.2 - 37.8	(-)10.2 - 37.8
Mass ratios of saponifiable fractions			
Free fatty acid	$0.20^{a} \pm 0.04$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
Monoacylglycerol	$0.30^{a} \pm 0.05$	$0.10^{b} \pm 0.00$	$0.30^{a} \pm 0.01$
Diacylglycerol	$0.00^{c} \pm 0.01$	$0.20^{a} \pm 0.01$	$0.10^{b} \pm 0.02$
Triacylglycerol	$0.50^{c} \pm 0.00$	$0.70^{a} \pm 0.01$	$0.60^{b} \pm 0.01$
Rice phytochemicals			
γ-oryzanol (mg/100g)	$840.00^{b} \pm 0.02$	$1030.00^{a} \pm 0.01$	$1030.00^{a} \pm 0.03$

Mean values in the same row followed by different superscripts are significantly different ($p < 0.05$).

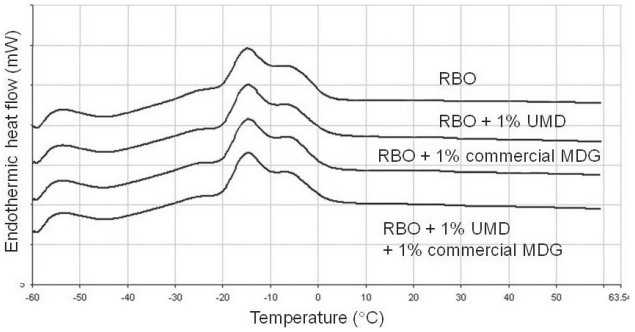

Fig. 3 Effect of surfactant addition on the melting profiles of rice bran oil (RBO). The surfactants used were the unevaporated fraction after molecular distillation at 140℃ (UMD) and commercial mono- and diacylglycerol (MDG).

cal composition and melting point. The melting point range of UMD was between (-)46.2 to 37.8℃ (Table 2); while the melting point range of commercial MDG was between 45-65℃ (results not shown). The melting temperature of RBO, in the absence or presence of surfactants, lay within a range of (-)30 to 5℃. This was due to the low concentration of the surfactant within 1-2%, which was lower than the concentration used to induce nucleation and crystallization of the surfactants[14]. Such low concentration was selected due to food additive regulation for the use of surfactants in fat products of not exceeding 1%[22].

From practical standpoints, physically refined RBO was prone to clouding when stored at low temperatures (15 and 25℃) as in the refrigerator or in temperate zone, observed as the solid fat content (SFC) at low temperature (Table 3). Although the melting profiles of RBO supplemented with UMD and commercial MDG were not significantly different, addition of UMD and MDG slightly increased SFC of RBO at 15℃ ($p<0.05$). The presence of MDG slightly increased the SFCs of RBO at 25℃ ($p<0.05$), indicating that commercial surfactant could induce nucleation of solid fat crystal shown as clouding defect in liquid oil. However, such crystallization of solid fat was too low to form fat crystal network between15-35℃.

The addition of UMD did not affect the apparent viscosity (at 25℃) of RBO and RBO-AMF blend at the ratio of 75:25. However, the addition of commercial MDG increased the apparent viscosity of RBO and RBO-AMF slightly ($p<0.05$; Table 4). This suggested that the solid fat particles induced by commercial MDG, despite minute amount, could induce nucleation that resisted liquid flow of RBO. Although the presence of UMD caused a minute amount of SFC to be retained at 25℃ (Table 3), it did not affect the viscosity of RBO at SFC similar to that of MDG (Table 4). The influences of both surfactants on solid fat particle, after aging at 5℃ and storage at 30℃ were then investigated further.

3.3 Effect of aging regime on solidification of RBO and RBO-AMF with added surfactants

The influence of UMD and commercial MDG on the solidified fat phase at a normal room temperature range in Thailand (25-30℃) was reported (Table 5). The storage stability investigation was carried out after different aging time at 5℃, which was the temperature close to melting

Table 3 Effect of surfactant addition on the onset of melting (*To*), end of melting temperarture (*Te*) and solid fat content (SFC) of rice bran oil (RBO). Surfactants used were the unevaporated fraction after molecular distillation at 140 ℃ (UMD) and commercial mono- and diacylglycerol (MDG).

Treatments	RBO	RBO+1% UMD	RBO +1% commercial MDG	RBO+ 1% UMD + 1% commercial MDG
To (℃)	(-)30.6^{a} ± 1.0	(-)28.5^{a} ± 1.2	(-)28.6^{a} ± 1.1	(-)27.7^{a} ± 1.6
Te (℃)	4.9^{a} ± 0.7	4.9^{a} ± 0.3	5.4^{a} ± 0.6	5.0^{a} ± 0.4
SFC at 15℃ (%)	0.68^{c} ± 0.01	0.77^{b} ± 0.06	0.84^{ab} ± 0.05	0.88^{a} ± 0.05
SFC at 25℃ (%)	0.39^{b} ± 0.04	0.48^{ab} ± 0.08	0.58^{a} ± 0.04	0.56^{a} ± 0.06
SFC at 35℃ (%)	0.12^{a} ± 0.02	0.11^{a} ± 0.03	0.13^{a} ± 0.02	0.13^{a} ± 0.02

Mean values (± standard deviation) in the same row followed by different superscripts are significantly different ($p < 0.05$).

Table 4 Effect of surfactant addition on apparent viscosity at 25℃ of rice bran oil (RBO) and rice bran oil–anhydrous milk fat (RBO-AMF) blended at a ratio of RBO to AMF of 75:25. Surfactants used were the unevaporated fraction after molecular distillation (UMD) obtained at 140℃ and commercial mono- and diacylglycerol (MDG).

Treatments	Apparent viscosity (mPa·s)
RBO	79.2^{b} ± 3.5
RBO + 1% UMD	75.9^{b} ± 1.7
RBO + 1% commercial MDG	88.6^{a} ± 3.2
RBO-AMF	75.9^{b} ± 9.4
RBO-AMF + 1% UMD	73.6^{b} ± 2.3
RBO-AMF + 1% commercial MDG	85.6^{a} ± 2.8

Mean values (± standard deviation) followed by different superscripts are significantly different ($p < 0.05$).

Table 5 Effect of aging and surfactant addition on oil-holding capacity at 30℃ under quiescent condition of rice bran oil (RBO) and rice bran oil–anhydrous milk fat (RBO-AMF) blended at a ratio of RBO to AMF of 75:25. Surfactants used were the unevaporated fraction after molecular distillation at 140℃ (UMD) and commercial mono- and diacylglycerol (MDG).

Types of oil blend	% Volume of solid fat phase					
	Aged at 5℃, 12 h			Aged at 5℃, 24 h		
	Before storage at 30℃	Storage at 30℃ for 12 h	Storage at 30℃ for 24 h	Before storage at 30℃	Storage at 30℃ for 12 h	Storage at 30℃ for 24 h
RBO	0.0^{b} ± 0.0	0.0^{c} ± 0.0	0.0^{c} ± 0.0	0.0^{b} ± 0.0	0.0^{c} ± 0.0	0.0^{c} ± 0.0
RBO + 1% UMD	0.0^{b} ± 0.0	0.0^{c} ± 0.0	0.0^{c} ± 0.0	0.0^{b} ± 0.0	0.0^{c} ± 0.0	0.0^{c} ± 0.0
RBO +1% commercial MDG	100.0^{a} ± 0.0	17.6^{b} ± 2.8	17.6^{b} ± 2.8	100.0^{a} ± 0.0	46.4^{b} ± 3.9	34.5^{b} ± 2.9
RBO-AMF	100.0^{a} ± 0.0	0.0^{c} ± 0.0	0.0^{c} ± 0.0	100.0^{a} ± 0.0	0.0^{c} ± 0.0	0.0^{c} ± 0.0
RBO-AMF +1% UMD	100.0^{a} ± 0.0	0.0^{c} ± 0.0	0.0^{c} ± 0.0	100.0^{a} ± 0.0	0.0^{c} ± 0.0	0.0^{c} ± 0.0
RBO-AMF +1% commercial MDG	100.0^{a} ± 0.0	89.4^{a} ± 11.1	92.9^{a} ± 5.4	100.0^{a} ± 0.0	93.8^{a} ± 6.9	92.5^{a} ± 7.1

Mean values (± standard deviation) in the same column followed by different superscripts are significantly different ($p < 0.05$).

temperature of RBO. After aging at 5℃, the aged RBO, both in the absence or presence of UMD, melted thoroughly (Table 5), since the temperature was the end temperature (*Te*) of crystallized RBO (Table 3). Prolonging aging time at 5℃ from 12 h to 24 h did not help structure-forming process of rice MDG in UMD to entrap liquid RBO. This is likely due to the similarity of oleyl ester of rice MDG, which were close to that of major fatty acid in RBO (Table 1). Thus, the rice MDG in UMD did not separate its phase to form self-assembled surfactant network during aging.

However, when the commercial MDG was used as structurant at 1%, it was found that the RBO-commercial MDG and RBO-AMF-commercial MDG blends remained solidified at 5℃ prior to storage. This was due to both fat crystal networks generated by MDG and/or AMF. After storage at 30℃ for 12 h, all samples melted although to the different degree.

Solidified RBO-commercial MDG blend aged at 5℃ for 12 h could, however, retain the volume of solid fat phase of 17% after storing at 30℃, although the RBO contained mostly unsaturated fatty acid (Table 1). A longer aging time at 5℃ of 24 h resulted in a more stable network than an aging time of 12 h. This was due to the presence of commercial MDG, which initiated fat crystal formation or seeding in RBO during aging at 5℃, as well as the formation of self-assembled surfactant network. Such network could stabilize solidified structure of RBO-MDG blend to some extent at 30℃ up to 24 h although the MDG was present at a low concentration of 1%. Prolonging aging time at 5℃ from 12 h to 24 h even enhanced the stability of solidified RBO at 30℃ to the higher extent.

The commercial MDG, which had low hydrophile-lipophile balance (HLB) as 1, could form spontaneous reverse bilayer in pure oil system due to its amphiphilic structure of polar head and hydrophobic alkyl chain[8]. Therefore, after cooling and aging, it was possible that the β-crystal network of MDG could be formed[8]. However, the structure forming process, physical and microstructural properties of MDG network in edible oil are depended on the concentration of self-assembled surfactants, temperature, aging process, cooling rate and shear rate[13, 14]. Nontheless, the self-assembled MAG structure was also reported to entrap cod liver oil in three-dimensional network when used at 5%[23]. The three-dimensional network forming process of RBO and RBO-high melting fat blends containing surfactants, however, required further in-depth investigation due to the complexity and compatibility of TAG and surfactant networks.

When AMF was blended with RBO to alter the contents and types of saturated fatty acids to enhance segregation of fat crystal and self-assembling process of MDG, it was found that the solid fat phase was stabilized and the volume of solid phase was retained at 100% in solidified RBO-AMF blend prior to storage at 30℃. However, when the aged RBO-AMF blends, both in the absence or presence of UMD, were stored at 30℃, they melted thoroughly despite the increased proportion of saturated fatty acids from AMF. This corroborated that the acyl group of surfactants played an important roles in initiating nucleation and fat crystal network during aging rather than the acyl groups in bulk oil phase. Otherwise the fat crystal network of RBO-AMF would have been retained after storage at 30℃. The melting point of AMF was within the range of 31 - 34℃ (according to manufacturer's datasheet). At 30℃, AMF alone could retain SFC around 45%[24].

The results of this study suggested that although the surfactants, at a level of 1–2% of bulk oil phase, did not significantly affect the DSC melting profiles of RBO, they could affect other thermo-physical properties of both RBO and RBO-AMF after the oils had been aged in their presence. The addition of commercial MDG to RBO-AMF could stabilize the solid fat network that held the liquid oil. This is due to the ability of commercial MDG to induce nucleation of solid fat crystals, as well as the formation of self-assembled network during aging at 5℃, that stabilized the solidified structure of RBO-AMF blends at 30℃.

For the case of RBO, the self-assembled commercial MDG may, in part, be responsible for the solidification of RBO after aging at 5℃ for at least 12 h. This was due to the molecular rearrangement during aging[13]. Such influence of commercial MDG could be used in the fabrication of *trans*-free solidified vegetable oil at 30℃ as the replacer for hydrogenated edible fat.

Rice UMD, however, was not able to stabilize the fat crystal network of aged RBO-AMF blends. This was probably due to the presence of rice MDG in the UMD, whose structure was most likely esterified by oleic acid. Such molecular structure, which was rich in the acyl group close to the major esterified fatty acid in RBO, does not favor fat crystallization and the formation of self-assembled surfactant network. Therefore the presence of UMD prevented network formation even in oil blends containing saturated fat from AMF.

It is apparent that fat crystal network of surfactants having different molecular acyl structures from those of bulk oil phase could help structuring solidified fat provided that the mixture was aged for 12-24 h at the temperature close to melting temperature of bulk oil phase. The commercial MDG, which had palmitic acid esterified mainly, could phase separate from the bulk oil phase of RBO, which contained oleic acid as major fatty acid. Phase separation of commercial MDG thus led to the formation of self-assembled crystalline surfactant, that functioned as an organogelator for the solidification of RBO and RBO-AMF blends after aging[13, 14].

4 CONCLUSIONS

The operating temperature during MD process influenced the contents of FFA, MAG, DAG and TAG but not γ-oryzanol. Aging of RBO and RBO-AMF mixed with commercial MDG at 5℃ for at least 12 h could stabilize solid structure of RBO and RBO-AMF blends at 30℃ to some extent. The rice MDG in UMD, however, was able to destabilize the solid structure in RBO-AMF blend, resulting in its liquid state at 30℃ although the blend contained 38.54% saturated fatty acids. This study also implicated that the presence of 1% MDG surfactant and aging regime could be used to modify solid structure of RBO and RBO-AMF blend provided that aging process at 5℃ for at least 12 h was involved in structure-forming process.

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