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Physicochemical properties of peanut oil-based diacylglycerol and their derived oil-in-water emulsions stabilized by sodium caseinate

Zhao Long [a,b], Mouming Zhao [a], Ning Liu [a,c], Daolin Liu [a], Dongxiao Sun-Waterhouse [a,d], Qiangzhong Zhao [a,*]

[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China
[b] College of Food Science and Engineering, Central South University of Forestry and Technology, Changsha 410004, China
[c] College of Life Science and Engineering, Shaanxi University of Science and Technology, Xi'an 710021, China
[d] School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand

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ABSTRACT

High purity peanut oil-based diacylglycerol (PO-DAG) (94.95 wt%) was prepared via enzymatic glycerolysis from peanut oil (PO). The resulting dominance of DAGs was proven to greatly influence the properties of corresponding fresh or frozen–thawed emulsions. Stable fresh oil-in-water emulsions were produced using either PO-DAG or PO, with stability enhanced by increased concentrations of Na-CN. The lower equilibrium interfacial tension along with greater negative ζ-potential of PO revealed that Na-CN was preferentially adsorbed to the PO interface. Adding 0.05 mol/L NaCl to the PO emulsions minimized depletion flocculation caused by the unadsorbed Na-CN, but further NaCl addition increased oil droplet size and concomitant coalescence. For the PO-DAG emulsions, adding 0.2 mol/L NaCl did not significantly ($p > 0.05$) affect their ζ-potential but adding 0.05 or 0.1 mol/L NaCl lowered ζ-potential, although NaCl at these concentrations increased oil droplet size and coalescence. Freezing–thawing process considerably weakened the stability of PO-DAG emulsions.

1. Introduction

Generally, animal fats or vegetable oils consist of triacylglycerols (TAGs) and small amounts of diacylglycerols (DAGs), monoacylglycerols (MAGs) and free fatty acids (D'alonzo, Kozarek, & Wade, 1982). These lipids are important food ingredients providing desirable sensory attributes and energy sources. Obesity has become a serious public health problem worldwide. Reduction in animal fat intake has been a direct and effective approach for combating weight gain and obesity as well as associated chronic diseases such as hypertension, hyperglycemia and cardiovascular diseases (Antipatis & Gill, 2001; Nejat, Polotsky, & Pal, 2010). Substitution of animal fats in foods with plant oil or derived products is one commonly used strategy.

Diacylglycerol, an ester of glycerol with two hydroxyl groups esterified with fatty acids, is a natural component of various edible oils (D'alonzo, Kozarek, & Wade, 1982). DAG has been designated as generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA), and is believed to be digested in the same way as other edible oils (Morita & Soni, 2009), with advantages of decreasing postprandial serum TAG levels and body fat built-up (Maki et al., 2002; Saito, Tomonobu, Hase, & Tokimitsu, 2006).

DAGs can be produced from various oils (Liu et al., 2012; Saberi, Chin-Ping, & Oi-Ming, 2011; Saitou et al., 2012) and lard (Cheong, Zhang, Xu, & Xu, 2009). Nowadays, DAGs are widely used as food additives in food manufacture [e.g. antioxidant (Waraho et al., 2012) or emulsifier (Awad & Hamada, 2001)], and as a fat substitute in cooking, including frying and baking (Cheong, Tan, Long, Affandi Yusoff, & Lai, 2010; Li, Kimura, Endo, Maruyama, & Fujimoto, 2005). Emulsions are one of the most important applications of DAGs and derived products including stable DAG mayonnaise with phospholipase A_2-treated egg yolk (Kawai, 2004) and stable meat emulsion through partially replacing lard with lard-DAG (Miklos, Xu, & Lametsch, 2011). Water-in-oil emulsions with DAG can be emulsified more easily than those with TAG, possibly due to the presence of only one hydroxyl group in DAG (Shimada & Ohashi, 2003). Under the same conditions, DAG-in-water emulsions have less unfolded β-lactoglobulin than TAG-in-water emulsions (Sakuno, Matsumoto, Kawai, Taihei, & Matsumura, 2008). The advantageous effects of DAGs on the emulsions, especially the oil-in-water emulsions, motivated the present investigation.

* Corresponding author at: College of Light Industry and Food Sciences, South China University of Technology, No. 381, Wushan Road, Tianhe District, Guangzhou 510640, China. Tel./fax: +86 20 87113914.
E-mail address: qzzhao@scut.edu.cn (Q. Zhao).

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In this study, we aimed to investigate the potential of DAG in food emulsions systems. We produced peanut oil based-diacylglycerol (PO-DAG) from commercially available peanut oil (PO), via enzymatic glycerolysis, using phospholipase Lecitase® Ultra. PO was selected for its unique flavor, high popularity, and excellent oxidative stability. PO is rich in monounsaturated fatty acids, vitamin E, folate, protein, manganese and, in particular, unsaturated linoleic acid in the sn-2 position of triglycerides (which is nutritionally important as the fatty acids at sn-2 will be conserved during digestion, compared to the fatty acids at sn-1 and sn-3 that are released by pancreatic lipase) (Yoshida, Hirakawa, Tomiyama, Nagamizu, & Mizushina, 2005; Young, 1996). The differences between the emulsions prepared with PO and the emulsions with PO-DAG were compared in the presence of sodium caseinate (Na-CN). The effect of adding NaCl on the properties of such emulsions was also examined.

2. Materials and methods

2.1. Materials

Na-CN (95% dry weight protein) was supplied by New Zealand Milk Products (NZMP, Fonterra Co-operative Group Limited, New Zealand). Phospholipase Lecitase® Ultra was purchased from Novozymes (Copenhagen, Denmark). Oil Red O was purchased from AMRESCO (Ohio, USA). Glycerol was obtained from Tianjin Chemical Reagent Factory (Tianjin, China). Peanut oil (PO) and corn oil were purchased from a local supermarket in Guangzhou (China). All the chemicals or solvents for high performance liquid chromatography (HPLC) analysis were of HPLC grade and the other reagents were of analytical grade.

2.2. Preparation of peanut oil-based diacylglycerol (PO-DAG)

The reaction mixture consisting of PO and glycerol was subjected to glycerolysis reaction using phospholipase Lecitase® Ultra (E.C.3.1.1.32) in a solvent-free system under the following conditions: glycerol/PO mole ratio, 5:1; enzyme load, 60 U/g substrates; initial water content, 4 wt% of substrates; temperature, 40 °C; reaction time, 10 h. The reaction mixture was incubated in a water bath and stirred with a magnetic stirrer (300 rpm). After the incubation, the resulting mixture was centrifuged at 8000*g* for 10 min at 20 °C to yield the crude PO-DAG product.

The crude PO-DAG product was subjected to purification via two-step molecular distillation using a MD-S80 equipment (Handway Technology Foshan Co. Ltd., Foshan, China) with an evaporator and condenser of 0.066 m^2 and 0.05 m^2, respectively, spaced at 30 mm. The first step was to separate glycerol, free fatty acids and MAGs, while the second step segregated the DAGs from TAGs. The same conditions were applied to both steps: evaporator temperatures 175 °C and 240 °C, respectively, for the first and second step; other operational conditions were the same for both steps: evaporator vacuum, 0.5–1.0 Pa; roller speed, 300 rpm; condenser temperature, 40 °C; feed rate, 100 mL/h; feed temperature, 80 °C.

2.3. Acylglycerol composition analysis

The acylglycerol composition of PO and PO-DAG was determined by reversed-phase high performance liquid chromatography (RP-HPLC) using a Waters P600 pump with quaternary gradient system (Waters, Massachusetts, USA) and a 3300 evaporative light-scattering detector (ELSD, Alltech, USA) with an atmosphere compression pump (Tianjing, China). Separation of acylglycerol components was performed using a Purospher® STAR RP-18e column (250 mm × 4.6 mm i.d., particle size 5 μm) (Merck, Darmstadt, Germany). The mobile phases consisted of A (acetonitrile:acetic acid = 99.95:0.05, v/v) and B (dichloromethane), and the following gradient was used: 0–4 min 100% A; 4–12 min 90% A and 10% B; 12–15 min 70% A and 30% B; 15–19 min 20% A and 80% B; 19–31 min 80% A and 20% B; 31–36 min 90% A and 10% B; 36–39 min 100% A; 39–42 min 100% A. The injection volume was 10 μL and flow rate of 0.6 mL/min was used. The effluent was monitored by the ELSD with an evaporator temperature at 40 °C and the flow rate of the atmosphere at 1500 mL/min. HPLC–MS was used to aid the identification of the compounds in the samples, following the method of Zhong et al. (2009). HPLC analysis of samples was carried out in duplicate.

2.4. Fatty acid composition (FAC) analysis

FAC was determined as fatty acid methyl esters by gas chromatography–mass spectrometer (GC–MS). The preparation of fatty acid methyl esters followed the method of Wang et al. (2010) with modification. The GC–MS system was equipped with a Trace Ultra GC (Thermo Finnigan, San Jose, CA, USA), a Trisplus automated sampler, a quadrupole DSQ II MS, and a TR-5MS capillary column (30 m × 0.2 mm, 0.25 μm, Thermo Finnigan, San Jose, CA, USA). The injection volume was 1.0 μL, and the injector was in split mode with a split ratio of 100:1. The injection temperature and ion source temperature were set at 250 °C and 230 °C, respectively. The oven temperature was programmed as follows: held at 40 °C for 1 min, increased from 40 °C to 150 °C (10 °C/min), held at 150 °C for 2 min, increased from 150 °C to 220 °C at 10 °C/min and from 220 °C to 280 °C at 5 °C/min, and held at 280 °C for 3 min. The carrier gas (helium) flow rate was 1.0 mL/min. The system was equilibrated at 40 °C before sample injection. Mass spectra was recorded at three scans per second over a scanning range from 50 to 500 *m*/*z*. Chromatograms and mass spectra were evaluated using the Xcalibur™ software bundle version 2.0 (Thermo Finnigan, San Jose, CA, USA). Data are reported as the averages of two measurements.

2.5. Differential scanning calorimetry (DSC) analysis

A TA Q100-DSC thermal analyzer (TA Instruments, New Castle, Delaware, USA) was used to analyze (in duplicate) the thermal properties of PO and PO-DAG. Samples (6.0 mg) were weighted and sealed into an aluminum pan. An empty and sealed aluminum pan was used as a reference. To obtain the exotherm, the samples were heated from room temperature to 60 °C (20 °C/min), held for 5 min, and cooled to −100 °C at 5 °C/min. To obtain the endotherm, the samples were held at −100 °C for 5 min, then heated to 60 °C at 5 °C/min. Onset temperature (T_o) and offset temperature (T_f) were determined at the extrapolated leading edge of the exotherm or endotherm intersects with the baseline.

2.6. Solid fat content (SFC) analysis

The SFC of PO or PO-DAG was determined using a NMI20-analyst (Niumag Electric Corporation, Shanghai, China). The samples (3–4 mg) were poured into the NMR tubes and tempered in water bath with 60 °C for 30 min, cooled and held at 0 °C for 60 min. The SFC was determined at temperatures ranging from 5 to 60 °C (at 5 °C intervals) by equilibrating the NMR tubes at these temperatures for 30 min before measurements. All measurements were conducted in duplicate.

2.7. Interfacial tension

Interfacial tension of the Na-CN solution (1 wt%) adsorbed at the oil–water interface was measured as a function of time

following the pendant drop method using a DataPhysics OCA20 contact angle meter (DataPhysica Instruments, Germany). A drop of the Na-CN solution (−15 μL) was formed at the bottom of a straight capillary column using an automatic dosing system with the surrounding medium as either PO or PO-DAG. A charged coupled device (CCD) camera photographed the contour of the droplet, from which the tension values were calculated using the SCA20 software (DataPhysica Instruments, Germany).

2.8. Emulsion preparation

Emulsions were prepared with oil (PO or PO-DAG, 10 wt%), water and Na-CN (either 1 wt% or 2 wt%). Aqueous Na-CN solutions (1 or 2 wt%) were first prepared with deionized water at neutral pH and 60 °C with stirring at a moderate shear rate until complete dissolution. Then PO or PO-DAG was added to the Na-CN solution. Coarse emulsions were then achieved through homogenization with an IKA T25 digital Ultra-Turrax disperser (IKA Works Inc., Wilmington, NC, USA) at 10,000 rpm for 1 min before further homogenization using a 2-stage single-piston homogenizer (APV-1000, Albertslund, Denmark) at 20 MPa (10% of total pressure over the second valve). To investigate the influence of ionic strength on the properties of emulsions, different amounts of NaCl (i.e. the final NaCl concentration was 0, 0.05, 0.1 or 0.2 mol/L) were added to the dispersions before premixing. The emulsions were subjected to analyses after a storage at 25 °C for 2 h (termed "fresh emulsion"), or being frozen at −18 °C for 24 h after being thawed at 25 °C for 4 h (termed "frozen–thawed emulsion").

2.9. Droplet size distribution

A Malvern MasterSizer 2000 (Malvern Instruments Co. Ltd., Worcestershire, UK) was used to determine the droplet size distribution and average diameter of the droplets in the fresh or frozen–thawed emulsions. The refractive index and adsorption of the dispersed phase were set at 1.414 and 0.001, respectively, and the refractive index of the continuous phase was at 1.330 (Long, Zhao, Zhao, Yang, & Liu, 2012). The emulsion in the sample chamber was diluted 1000-fold with deionized water. Volume weighted average diameter ($d_{4,3}$, μm), was calculated using the following equation:

$$d_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3 \quad (1)$$

where d_i is the particle size, and n_i is the number of particles with diameter d_i.

2.10. ζ-potential measurement

Zeta-potential measurement of the fresh emulsions was carried out by laser Doppler velocimetry and dynamic light scatting technique using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Co. Ltd., Worcestershire, UK) at 25 °C. Samples were diluted with deionized water until the protein content was 0.2 mg/mL. One milliliter of each diluted sample was put in a visibly clear disposable zeta cell (Model DTS 1060C, Malvern Instruments Co. Ltd., Worcestershire, UK) without any air bubbles. The equilibrium time was 1 min.

2.11. Microscope observation

Microscopic images were observed under Olympus CX31 light microscope (Olympus, Tokyo, Japan) equipped with a digital camera MShot MD130 (Guangzhou Mingmei Technology Co., Ltd., Guangzhou, China). Samples were placed on a clean glass slide and covered with a cover slip for microscopic observation (total magnification 400×, objective lens: 40×, eyepiece lens 10×). Images were processed by software Micro-Shot Basic version 1.0 (Guangzhou Mingmei Technology Co., Ltd., Guangzhou, China).

2.12. Partial coalescence of fat

The partial coalescence of fat was determined by the method of Palanuwech, Potineni, Roberts, and Coupland (2003). Oil Red O solution (0.0015 wt% in oil) was prepared using corn oil with gentle stirring for at least 12 h to allow complete dispersion. A mixture consisting of 20 g emulsion and 10 g Oil Red O solution was added to a 50 mL centrifuge tube before centrifugation at 10,000*g* and 30 °C for 30 min using a temperature-controlled centrifuge (Model GL-21 M, Xiangyi Instrument Co. Ltd., Changsha, China). The diluted-dye solution fraction was transferred carefully from the surface, and the absorbance was measured at 520 nm using a UV–visible spectrophotometer (Perkin-Elmer, Lambda 3, Norwalk, CT). Corn oil was taken as the blank. The change in absorbance indicated the mass of fraction that was not emulsified in the fat (φ_d), which was calculated as:

$$\varphi_d = m_o(a-1)/(m_e\phi) \quad (2)$$

where m_o is the weight of added Oil Red O solution, m_e is the weight of emulsion, a is the ratio of absorbance of Oil Red O solution before and after centrifugation, and ϕ is the mass fraction of oil in the emulsion.

2.13. Statistical analysis

Statistical analysis was performed using SPSS 11.5 (SPSS Inc., Chicago, IL) with one-way ANOVA. Student–Newman–Keuls test was used for comparing the mean values among treatments, and identifying the significance of difference ($p < 0.05$) among treatments.

3. Results and discussion

3.1. Acylglycerol composition and FAC of PO and PO-DAG

The acylglycerol composition and FAC of PO and PO-DAG are summarized in Table 1. The PO had 0.40 wt% DAG. Enzymatic glycerolysis and purification via molecular distillation led to the increase of DAG concentration to 94.95 wt%., a simultaneous and

Table 1
Acylglycerol composition and fatty acid composition of peanut oil (PO) and peanut oil-based diacylglycerol (PO-DAG).[a]

	PO	PO-DAG
Acylglycerol compositions (wt%)		
MAG	0.22	0.96
DAG	0.40	94.95
TAG	99.38	4.09
Fatty acid composition (wt%)		
C16:0	13.52	13.80
C18:0	3.24	4.51
C20:0	1.39	1.64
C22:0	1.50	2.39
C24:0	1.10	1.08
C18:1	45.82	41.33
C18:2	28.63	33.93
C20:1	4.79	1.33
Unsaturated fatty acid	79.24	76.59
Saturated fatty acid	20.76	23.41

PO, peanut oil; PO-DAG, peanut oil-based diacylglycerol; MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol.
[a] Values show the means of two replicates.

remarkable reduction of the TAG content from 99.38 wt% to 4.09 wt%, and a slight increase of MAG content from 0.22 wt% to 0.96 wt%.

Both PO and PO-DAG had a high concentration of long chain unsaturated fatty acids (UFA) including oleic acid (C18:1; 45.82% and 41.33%, respectively) and linoleic acid (C18:2; 28.63% and 33.93%, respectively). The combined enzymatic glycerolysis and purification processes did not change significantly the pattern of PO and PO-DAG fatty acid (FA) profiles, i.e. only some variations detected in the contents of individual FAs. PO-DAG had slightly more UFAs and slightly less SFAs than PO. The reduction of UFA in PO-DAG was attributed to the small decrease in oleic acid (C18:1) and eicosenoic acid (C20:1), although a small increase occurred to C18:2. There was also an increase in the total SFAs, due to the elevated C18:0 and C22:0 contents (while other SFAs remained unchanged).

3.2. DSC heating and cooling profiles and SFC of PO and PO-DAG

DSC is commonly used to evaluate the thermal behaviors of substances such as oil products and polymers. Crystallization and melting thermograms of PO and PO-DAG examined by DSC are shown in Fig. 1a and b, respectively. The transition temperatures of crystallization and melting points are summarized in Table 2, including onset temperature (T_o), offset temperature (T_f) and temperature range (difference between T_o and T_f). The crystallization profile of PO (T_o −2.19 °C; T_f −63.93 °C) exhibited four exothermic peaks (Fig. 1a). The onset crystallization peak (peak 4, −3.94 °C) was relatively steep, indicating initial nucleation over a short period of time. The three overlapping peaks with low intensity (peak 2, 3, 4) suggest a low growth of crystal structure. PO exhibited a distinct broadening exotherm peak at −63.92 °C (Table 2). The crystallization regions of the DSC crystallization plot for PO-DAG appeared to shift to higher temperatures, i.e. T_o and T_f to 18.70 °C and −56.60 °C, respectively, with a major sharp peak (peak 2, −17.11 °C) and a shoulder (peak 3, −10.23 °C) indicating acceleration of nucleation and large crystal structure in this region.

Fig. 1b shows the melting curves of PO and PO-DAG. For PO, a major endotherm peak (peak 2, −17.26 °C) with two shoulder peaks at higher temperature (peaks 3 and 4) and a small fusion peak at a lower temperature (peak 1) were found. The melting curve of PO-DAG showed two major coordinate peaks with a transition temperature of −2.21 °C (peak 3) and 11.97 °C (peak 4), respectively. The endotherm regions at the lower temperature contained two small fusion peaks, while the endotherm regions at the higher temperature followed by a distinct endothermic peak. Interestingly, the T_o obtained from the melting curve of PO-DAG (−41.83 °C) was similar to PO (−41.97 °C). The T_f was clearly elevated from 11.45 °C to 28.31 °C after enzymatic glycerolysis, which agreed with the results of crystallization. As indicated by Tan and Man (2000), it is not easy to interpret the complex melting curves, because the cooling thermogram is only affected by the chemical composition of oil, but the melting thermogram is much more complex as a consequence of polymorphism of oils and fats.

In general, PO-DAG had exotherm and endotherm peaks over a wider temperature range, with the crystallization and melting regions being shifted to higher temperatures. The differences in the DSC thermogram between PO and PO-DAG could result from

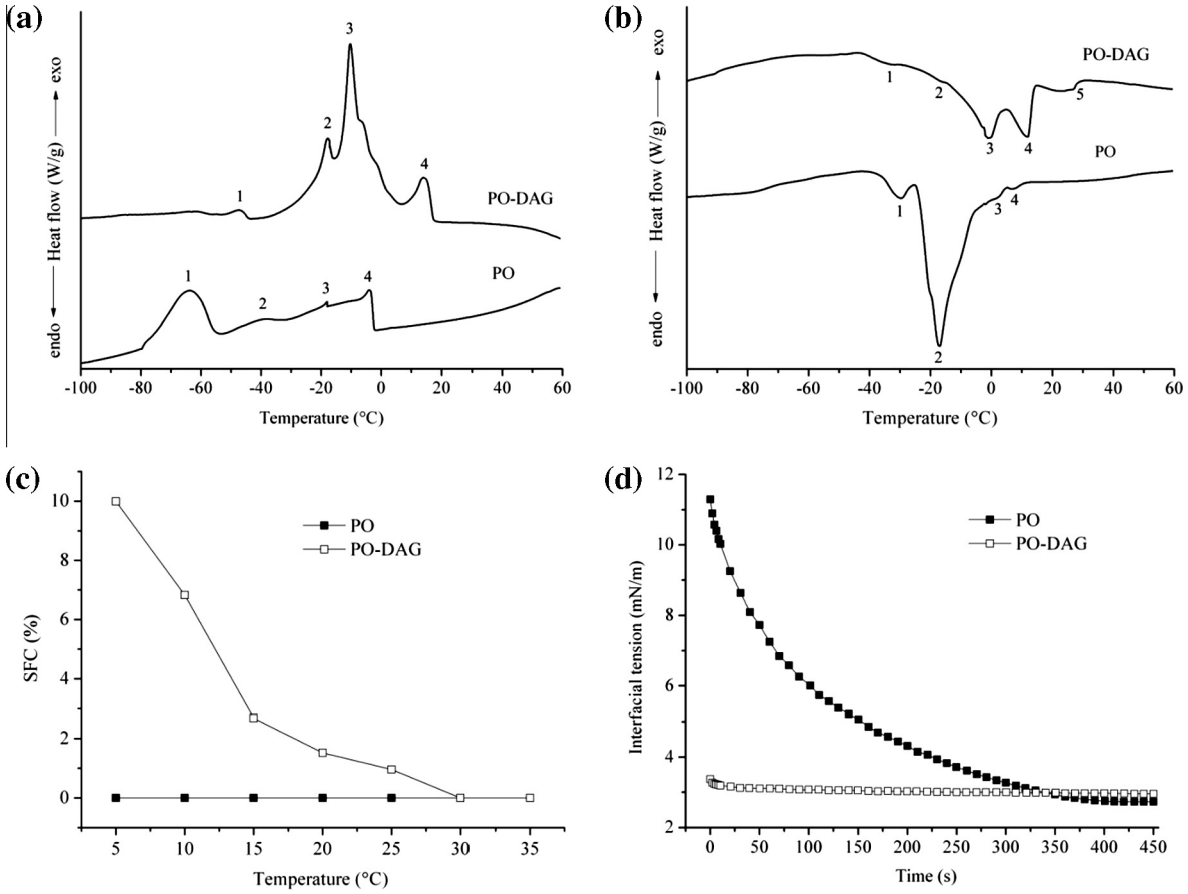

Fig. 1. DSC crystallization (a) and melting (b) thermograms, and solid fat content (SFC) versus temperature plots (c) and the plots of interfacial tension versus time for the 1 wt% sodium caseinate solution (d) in the presence of peanut oil (PO) or peanut oil-based diacylglycerol (PO-DAG).

Table 2
The onset, offset and transition temperatures derived from the crystallization curves of PO and PO-DAG by DSC.[a]

Curve	Sample	T_o (°C)	T_f (°C)	Temperature range	Transition temperature (°C)				
					1	2	3	4	5
Crystallization	PO	−2.19	−63.93	61.74	−63.92	−40.47	−17.97	−3.94	
	PO-DAG	18.70	−56.60	75.30	−47.22	−17.11	−10.23	14.44	
Melting	PO	−41.97	11.45	53.42	−30.04	−17.26	−2.17	7.55	
	PO-DAG	−41.83	28.31	70.14	−33.90	−14.62	−2.21	11.97	22.73

PO, peanut oil; PO-DAG, peanut oil-based diacylglycerol, T_o: onset temperature, T_f: offset temperature.
[a] The values show the means of two replicates.

the higher SFA content of PO-DAG and the different structures of acylglycerols (Table 1).

SFC, the quantity of fat crystals in a fat or fat blend, gives an indication of the suitability of a fat for a particular application, e.g. SFC can be used as an indicator for fat spreadability, resistance to oil-off, mouth feel and flavor release properties (Dian, Sundram, & Idris, 2007). The plots of SFC as a function of temperature for PO and PO-DAG differed remarkably (Fig. 1c). The SFC plot for PO-DAG was not linear whilst PO behaved like a liquid within the range of temperatures studied (5–60 °C) due to its high proportion of UFA. The conversion of PO to PO-DAG resulted in a considerably higher initial SFC for PO-DAG (i.e. ~10 times as high as that of PO at 5 °C). The largest decline in SFC of PO-DAG occurred from 5 to 15 °C, possibly due to the large proportion of DAG solubilized within this temperature range. The PO-DAG was completely solubilized when temperature reached 30 °C. The higher SFC for PO-DAG could be attributed to its higher SFA and lower UFA contents, although the impact of different acylglycerol structures should not be excluded.

3.3. Interfacial tension at the oil/water phase boundary between Na-CN and PO or PO-DAG

Examining interfacial tension at the interface is very important for understanding the adsorption behavior of Na-CN at the oil/water interface where PO and PO-DAG acted as the oil phase. The interfacial tension of PO and PO-DAG against 1 wt% Na-CN solution as a function of time is plotted in Fig. 1d. The interfacial tension of PO against the Na-CN solution had an initial value of 11.3 mN/m, and then declined at a greater and smaller rate, respectively, before and after 150 s, until an equilibrium value of about 2.73 mN/m at the 400th s. In comparison, PO-DAG, had an almost constant interfacial tension of 3.37 mN/m over 450 s. PO-DAG and PO were dominant by DAGs and TAGs, respectively, and PO-DAG has a relatively high MAG content (Table 1). DAGs have excellent interfacial activity because of their hydrophobic glycerol moiety and a hydrophilic moiety derived from the free hydroxyl group. In general, TAG renders about twice as much interfacial tension as DAG (Saberi, Kee, Oi-Ming, & Miskandar, 2011). Thus, it is understandable that PO-DAG had a much lower initial interfacial tension, although PO-DAG had a slightly higher interfacial tension (2.96 mN/m) than PO after the plateau (equilibrium) was achieved. Wang et al., 2012 suggested that increasing oil polarity would led to a decrease in molecular interactions between protein and oil. It could be assumed the amount of Na-CN adsorbed at PO-DAG-water interface was lower due to the higher polarity of PO-DAG compared to PO. Therefore, the equilibrium interfacial tension was found to be higher.

3.4. ζ-potential of emulsion

Fig. 2 shows the ζ-potential of fresh PO or PO-DAG emulsions prepared with different amounts of NaCl (0–0.2 mol/L). All samples exhibited negative ζ-potential (attributed to negatively charged

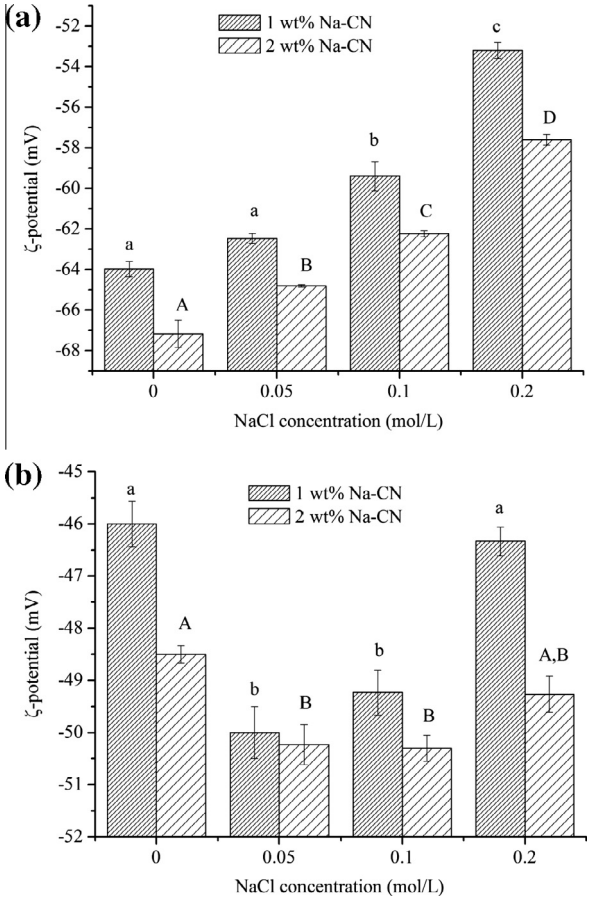

Fig. 2. The effects of sodium caseinate (Na-CN) or NaCl on the ζ-potential of peanut oil (PO) (a) or peanut oil-based diacylglycerol (PO-DAG) (b) emulsions. Error bars refer to standard deviations ($n = 3$). Different lowercase or uppercase letters indicate significant difference ($p < 0.05$) between the emulsions stabilized by 1 wt% and 2 wt% Na-CN, respectively, at different NaCl concentrations.

Na-CN) and were lower than −30 mV, thus indicating good stability of all the analyzed emulsions. The emulsions stabilized with 2 wt% Na-CN were more negative than those with 1 wt% Na-CN. As expected, the ζ-potential of the PO emulsions became less negative when NaCl concentration was increased within the range of 0–0.2 mol/L due to electrostatic screening effects (Guzey & McClements, 2007). Addition of NaCl at 0.2 mol/L could increase the ζ-potential by 10.8 and 9.6 mV, respectively, for the emulsions stabilized with 1 wt% and 2 wt% Na-CN.

For the PO-DAG emulsions with 1 wt% or 2 wt% NaCl, there was no significant difference in ζ-potential between the emulsions with zero and 0.2 mol/L NaCl, and between the emulsions with 0.05 and 0.1 mol/L NaCl (Fig. 2b). The emulsions containing 0.05 and 0.1 mol/L NaCl showed more negative ζ-potential. A higher Na-

CN content in the PO-DAG emulsion also led to more negative ζ-potential, a result resembled the PO emulsions. In general, the PO-DAG emulsions had higher (less negatively) absolute ζ-potential values. The differences in ζ-potential resulted from the state of adsorbed protein rather than the components of TAG and DAG (Sakuno et al., 2008). A decreased ζ-potential indicates reduced electrostatic repulsion between the oil droplets. Our results further indicate that Na-CN favors adsorbing to the PO-water interface. A higher surface concentration of Na-CN to PO emulsions was then confirmed, based on the interfacial tension data and the slight difference in the MAG content between PO and PO-DAG.

3.5. Average droplet size ($d_{4,3}$) of emulsion

The volume weighted average droplet size ($d_{4,3}$) of fresh and frozen–thawed PO or PO-DAG emulsions varied as a function of Na-CN and NaCl concentrations (Table 3). For the fresh PO emulsion with 1 wt% Na-CN, addition of NaCl up to 0.1 mol/L led to a gradual reduction in $d_{4,3}$ from 0.954 μm to 0.827 μm. Further increase of the NaCl concentration to 0.2 mol/L caused a slight increase in $d_{4,3}$ (i.e. 0.907 μm). For the fresh PO emulsion with 2 wt% Na-CN, the $d_{4,3}$ decreased from 0.885 μm to 0.771 μm on addition of 0.05 mol/L NaCl, but $d_{4,3}$ increased when NaCl was added at 0.1 or 0.2 mol/L. In comparison, the freezing–thawing process did not alter the $d_{4,3}$ changing pattern of the emulsions with 1 wt% Na-CN (i.e. all the $d_{4,3}$ values slightly increased). But for the 2 wt% Na-CN emulsions, the freezing–thawing process eliminated the difference in $d_{4,3}$ between those with 0 and 0.2 mol/L NaCl, and between those with 0.05 and 0.1 mol/L NaCl.

The $d_{4,3}$ values of the fresh PO-DAG emulsions were about half those of the fresh PO emulsions under the same conditions. The smaller droplet size for the PO-DAG emulsions possibly resulted from the high miscibility of PO-DAG with water due to the free hydroxyl groups in DAG, as well as the slightly higher MAG content in PO-DAG (which led to high surface activity). Addition of NaCl generally facilitated higher $d_{4,3}$ values for all the fresh and frozen–thawed PO-DAG emulsions. These results associated with PO-DAG differ from those for the PO emulsions. The PO-DAG emulsions stabilized by Na-CN at two different concentrations showed a similar trend although smaller droplets were observed for the emulsions with 2 wt% Na-CN. The $d_{4,3}$ of the PO-DAG emulsions increased greatly after the freezing–thawing process, suggesting a high instability of the emulsion. NaCl addition modified the behavior of emulsions, e.g. causing droplet aggregation and enhancing emulsion instability. NaCl addition affected the ionic strength of emulsions via shielding effects on the negative charges of the carboxylate head groups, thereby altering the nature of interface and reducing electrostatic repulsion. Such changes would then lead to different rates of coalescence depending on the type of oil phase (PO or PO-DAG). With the increasing ionic strength, the screening of charges would enable more compact packing and greater amounts of adsorbed caseins. Moreover, there existed a critical salt concentration for an emulsion of specific Na-CN concentration that contained limited numbers of carboxylate head groups. Further increase in the amount of NaCl above the critical concentration could alter the hydrogen bonding of water molecules and strengthened the hydrophobic attraction between droplets (Mitidieri & Wagner, 2002).

3.6. Microstructure

Protein-stabilized emulsions are normally susceptible to environmental changes especially changes in temperature. Thus, freezing–thawing stability is one of the critical quality factors for emulsions. Fig. 3a–d shows the effect of freezing–thawing on the microstructure of emulsions with/without adding 0.05 mol/L NaCl. In the absence of NaCl, the fresh PO emulsion containing 1 wt% Na-CN showed only slight aggregation, but freezing–thawing increased this aggregation resulting in large oil droplets. However, the presence of 0.05 mol/L NaCl diminished differences caused by the freezing–thawing process, i.e. more homogeneous emulsions were obtained with/without freezing–thawing (Fig. 3a). In the case of PO emulsion with 2 wt% Na-CN, flocculation was clearly observed in the absence of NaCl, and freezing–thawing remarkably promoted flocculation. The presence of 0.05 mol/L NaCl again diminished these differences caused by the freezing–thawing process (Fig. 3b). The flocculation was found reversible and some flocs could be disrupted by gentle stirring when NaCl concentration was <0.2 mol/L. Further increase of NaCl to 0.2 mol/L could generate big droplets due to coalescence (data not shown). These phenomena were supported by changes detected in the $d_{4,3}$ values, i.e. decrease followed by increase (Table 3).

The fresh PO-DAG emulsions with or without NaCl (0.05 mol/L) appeared to be homogeneous, with no aggregation or depletion flocculation at either Na-CN concentration (i.e. 1 wt% or 2 wt%) (Fig. 3c and d). This finding suggests that the fresh PODAG emulsions were stable. Due to higher miscibility with water, the PO-DAG emulsions could have tiny droplets and enormous surface area after homogenization. The freezing–thawing process led to the occurrence of oil clustering, coalescence, and formation of relatively large oil droplets in the PO-DAG emulsions with/without NaCl. The oil droplets were bigger in the 1 wt% Na-CN emulsions

Table 3
Volume weighted average diameter ($d_{4,3}$, μm) of PO or PO-DAG emulsions at different Na-CN and NaCl concentrations.[a]

NaCl concentration (mol/L)	Sodium caseinate concentration			
	1 wt%		2 wt%	
	Fresh	Frozen–thawed	Fresh	Frozen–thawed
PO				
0	0.954 ± 0.000^{a}	1.008 ± 0.008^{a}	0.885 ± 0.003^{a}	0.893 ± 0.003^{a}
0.05	0.861 ± 0.003^{c}	0.942 ± 0.003^{b}	0.771 ± 0.003^{d}	0.822 ± 0.003^{b}
0.1	0.827 ± 0.000^{d}	0.876 ± 0.003^{d}	0.784 ± 0.003^{c}	0.819 ± 0.000^{b}
0.2	0.907 ± 0.000^{b}	0.936 ± 0.000^{c}	0.867 ± 0.003^{b}	0.892 ± 0.007^{a}
PO-DAG				
0	0.467 ± 0.000^{A}	25.744 ± 0.6583^{A}	0.406 ± 0.000^{A}	8.725 ± 0.0550^{A}
0.05	0.481 ± 0.003^{B}	38.610 ± 0.8578^{B}	0.412 ± 0.000^{B}	17.310 ± 0.1794^{B}
0.1	0.497 ± 0.000^{C}	47.287 ± 0.3605^{C}	0.424 ± 0.003^{C}	21.366 ± 0.3276^{C}
0.2	0.519 ± 0.000^{D}	46.323 ± 0.2483^{C}	0.436 ± 0.000^{D}	21.035 ± 0.8256^{C}

Different lowercase or uppercase superscripts in the same column indicate significant difference ($p < 0.05$) between PO emulsions or between PO-DAG emulsions, respectively, at different NaCl concentrations. PO, peanut oil; PO-DAG, peanut oil-based diacylglycerol.

[a] Data are presented as means ± standard deviation ($n = 3$).

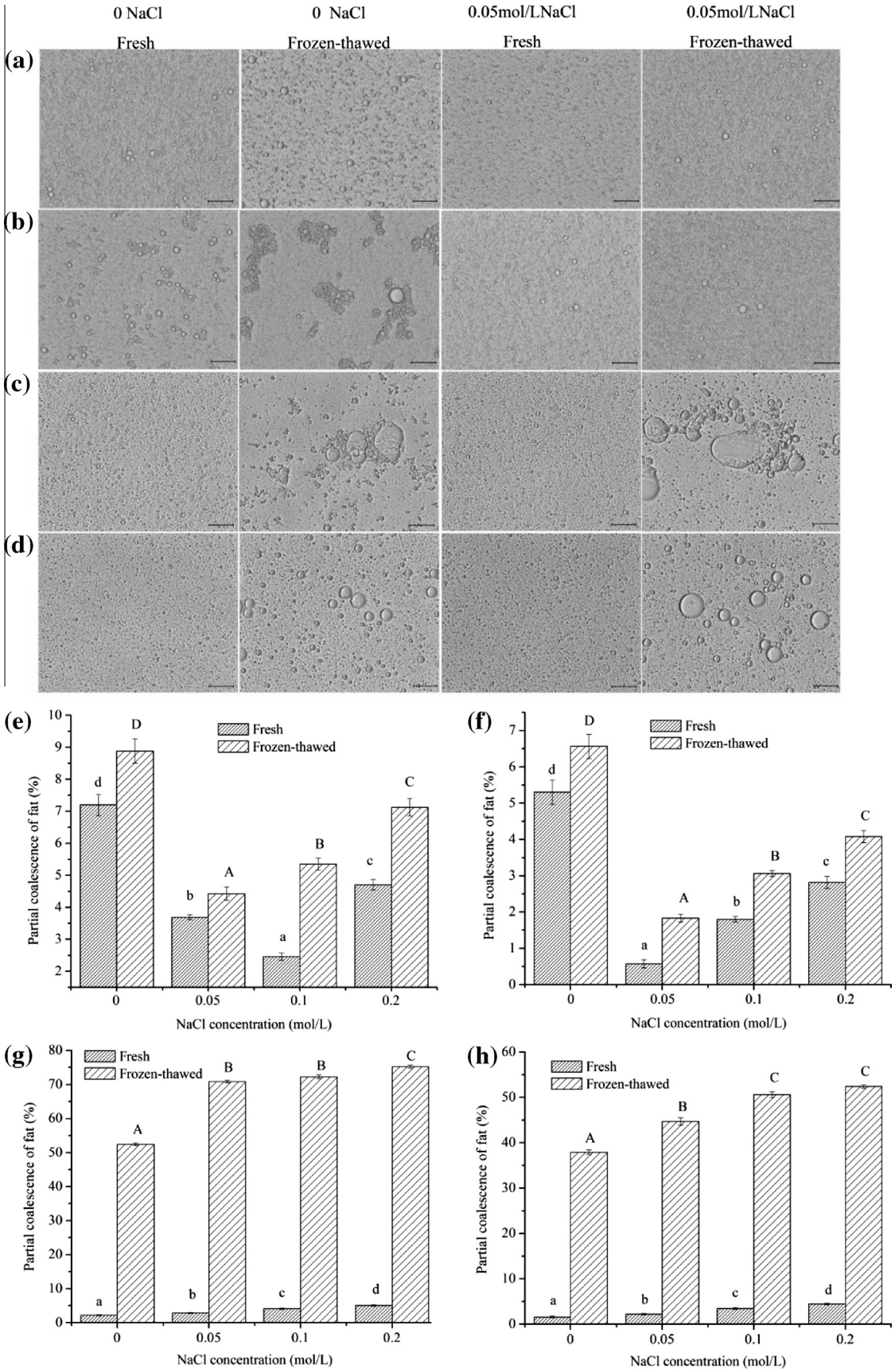

Fig. 3. Light microscopy images (a–d) and partial coalescence of fat (e–h) of the peanut oil (PO) and peanut oil-based diacylglycerol (PO-DAG) emulsions. (a, e) PO + 1 wt% sodium caseinate (Na-CN); (b, f) PO + 2 wt% Na-CN; (c, g) PO-DAG + 1 wt% Na-CN; (d, h) PO-DAG + 2 wt% Na-CN. In a–d, the scale bar represents 20 μm. In e–h, the heights of column and error bars represent the mean values and standard deviations, respectively ($n = 3$). Different lowercase or uppercase letters indicate significant difference ($p < 0.05$) between the fresh emulsions or between the frozen–thawed emulsions, respectively, at different NaCl concentrations.

than those in the 2 wt% Na-CN emulsions. Salt addition was responsible for elevated $d_{4,3}$ values of fresh PO-DAG emulsions (Table 3).

The depletion flocculation was mainly attributed to the unbonded or unadsorbed Na-CN. The presence of NaCl could increase the amount of Na-CN adsorbed. These findings were mostly in agreement with those of found by Srinivasan, Singh, and Munro (2000), in which adding NaCl increased the surface protein concentration and reduced gradually the extent of flocculation in the emulsions with 3% Na-CN. The difference between the PO-DAG and PO emulsions caused by the freezing–thawing resulted from the differences between PO-DAG and PO in crystallization and melting profiles and SFC. Moreover, low affinity of Na-CN to PO-DAG and slightly more MAGs (which are capable of fast adsorption) in PO-DAG could result in a weaker interfacial film consisting of Na-CN and MAG, rendering susceptibility to piercing by fat crystals. It is worth noting that although Na-CN adsorbed preferably onto the PO-water interface, the total amount of Na-CN adsorbed was higher for the PO-DAG emulsions than for the PO emulsion (data not shown).

3.7. Partial coalescence of fat

Fig. 3e–h further demonstrates the effects of Na-CN concentration and ionic strength on the partial coalescence of fat in fresh or frozen–thawed emulsions. The changing patterns between the fresh emulsions with PO and those with PO-DAG differed largely, and the magnitude of partial coalescence of fat in the frozen–thawed emulsions was about 10 times greater when using PO-DAG than using PO. The partial coalescence of fat for the fresh PO emulsions decreased with increasing NaCl from 0 to 0.1 mol/L and ascended when NaCl was at 0.2 mol/L NaCl. Such a changing pattern was in agreement with the $d_{4,3}$ values. The freezing–thawing process did not alter the pattern of change as a function of the NaCl concentration but increased the extent of partial coalescence of fat. Using Na-CN at 2 wt% Na-CN significantly suppressed the extent and altered the changing pattern of partial coalescence of fat, i.e. most severe partial coalescence of fat occurred to the emulsions without NaCl whilst a steady increase of partial coalescence of fat with increasing NaCl from 0.05 to 0.2 mol/L for both the fresh and frozen–thawed emulsions (Fig. 3e and f). For the PO-DAG emulsions, a steady increase in partial coalescence of fat with increasing NaCl concentration was found in all cases and, in particular, the frozen–thawed emulsions exhibited 10–18 times as much partial coalescence of fat as the fresh emulsions (irrespective of the Na-CN concentration). An increment in NaCl concentration from 0 to 0.2 mol/L increased the partial coalescence of fat in the fresh emulsions from 2.21% to 5.06% for 1 wt% Na-CN and from 1.58% to 4.43% for 2 wt% Na-CN, respectively. The partial coalescence of fat of the frozen–thawed emulsions made with 1 wt% or 2 wt% Na-CN increased from 52.4% to 75.2% and from 37.9% to 52.4%, respectively. Partial coalescence of fat was increased substantially after freezing–thawing, a result consistent with the findings by microscopy (Fig. 3c and d). The interfacial membrane formed by Na-CN and MAGs in the PO-DAG emulsion appeared to be fragile and could easily be penetrated by fat crystal during freezing (Garofalakis & Murray, 2001; Golding & Sein, 2004). Droplets that had been penetrated during freezing would fuse together, causing droplet clumps and coalescence during thawing. It was previously reported that partial coalescence of fat normally increased with an elevated SFC (Boode, Walstra, & Groot-Mostert, 1993). In this study, the SFC of PO-DAG was much higher than PO at temperature from 5 °C to <30 °C, therefore, it is anticipated that the partial coalescence of fat in the PO-DAG emulsions would be much more severe than in the PO emulsions after freezing–thawing.

4. Conclusions

High purity peanut oil-diacylglycerol (PO-DAG, 95%) was successfully produced via enzymatic glycerolysis and purification. The enzymatic glycerolysis used in this present study could modify the acylglycerol composition, FAC, SFC, melting behavior, crystallization and interfacial tension of PO, yielding PO-DAG with distinct composition and functional properties, i.e. the resulting dominance of DAG components, and a small, but significant, increase in MAGs (rather than initial dominance of TAGs in PO), and fresh or frozen–thawed emulsions with greatly altered physicochemical characteristics. It is feasible to use PO-DAG or PO to prepare stable fresh emulsions stabilized by Na-CN, given the small droplet sizes, homogeneous microstructure and low partial coalescence of fat in both type of emulsions. However, a freezing–thawing process could greatly weaken the stability of PO-DAG emulsion due to their fragile droplet interfacial membrane. Thus, the PO-DAG emulsion obtained in this study would be quiescent stable during storage and potentially exhibit good whippability (indicated by its partial coalescence of fat behavior), which indicates the potential of DAGs in the applications for whipping cream food products.

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References

Antipatis, V. J., & Gill, T. P. (2001). Obesity as a global problem. In P. Bjorntorp (Ed.), *The international textbook of obesity* (pp. 3–22). Chichester: Wiley.

Awad, T., & Hamada, Y. (2001). Effects of addition of diacylglycerols on fat crystallization in oil-in-water emulsion. *European Journal of Lipid Science and Technology, 103*, 735–741.

Boode, K., Walstra, P., & Groot-Mostert, A. E. A. (1993). Partial coalescence in oil-in-water emulsions 2. Influence of the properties of the fat. *Colloids and Surfaces A, 81*, 139–151.

Cheong, L.-Z., Tan, C.-P., Long, K., Affandi Yusoff, M. S., & Lai, O.-M. (2010). Physicochemical, textural and viscoelastic properties of palm diacylglycerol bakery shortening during storage. *Journal of the Science of Food and Agriculture, 90*, 2310–2317.

Cheong, L.-Z., Zhang, H., Xu, Y., & Xu, X. (2009). Physical characterization of lard partial acylglycerols and their effects on melting and crystallization properties of blends with rapeseed oil. *Journal of Agricultural and Food Chemistry, 57*, 5020–5027.

D'alonzo, R. P., Kozarek, W. J., & Wade, R. L. (1982). Glyceride composition of processed fats and oils as determined by glass capillary gas chromatography. *Journal of the American Oil Chemists Society, 59*, 292–295.

Dian, N. L. H. M., Sundram, K., & Idris, N. A. (2007). Effect of chemical interesterification on triacylglycerol and solid fat contents of palm stearin, sunflower oil and palm kernel olein blends. *European Journal of Lipid Science and Technology, 109*, 147–156.

Garofalakis, G., & Murray, B. S. (2001). Dilatational rheology and foaming properties of sucrose monoesters in the presence of β-lactoglobulin. *Colloids and Surfaces B, 21*, 3–17.

Golding, M., & Sein, A. (2004). Surface rheology of aqueous casein–monoglyceride dispersions. *Food Hydrocolloids, 18*, 451–461.

Guzey, D., & McClements, D. J. (2007). Impact of electrostatic interactions on formation and stability of emulsions containing oil droplets coated by β-lactoglobulin-pectin complexes. *Journal of Agricultural and Food Chemistry, 55*, 475–485.

Kawai, S. (2004). Characterization of diacylglycerol oil mayonnaise emulsified using phospholipase A_2-treated egg yolk. *Journal of the American Oil Chemists Society, 81*, 993–998.

Li, C. M., Kimura, F., Endo, Y., Maruyama, C., & Fujimoto, K. (2005). Deterioration of diacylglycerol- and triacylglycerol-rich oils during frying of potatoes. *European Journal of Lipid Science and Technology, 107*, 173–179.

Liu, N., Wang, Y., Zhao, Q. Z., Cui, C., Fu, M., & Zhao, M. M. (2012). Immobilisation of lecitase® ultra for production of diacylglycerols by glycerolysis of soybean oil. *Food Chemistry, 134*, 301–307.

Long, Z., Zhao, M. M., Zhao, Q. Z., Yang, B., & Liu, L. Y. (2012). Effect of homogenisation and storage time on surface and rheology properties of whipping cream. *Food Chemistry, 131*, 748–753.

Maki, K. C., Davidson, M. H., Tsushima, R., Matsuo, N., Tokimitsu, I., Umporowicz, D. M., et al. (2002). Consumption of diacylglycerol oil as part of a reduced-energy diet enhances loss of body weight and fat in comparison with consumption of a triacylglycerol control oil. *American Society for Clinical Nutrition, 76*, 1230–1236.

Miklos, R., Xu, X. B., & Lametsch, R. (2011). Application of pork fat diacylglycerols in meat emulsions. *Meat Science, 87*, 202–205.

Mitidieri, F. E., & Wagner, J. R. (2002). Coalescence of o/w emulsions stabilized by whey and isolate soybean proteins. Influence of thermal denaturation, salt addition and competitive interfacial adsorption. *Food Research International, 35*, 547–557.

Morita, O., & Soni, M. G. (2009). Safety assessment of diacylglycerol oil as an edible oil: A review of the published literature. *Food and Chemical Toxicology, 47*, 9–21.

Nejat, E. J., Polotsky, A. J., & Pal, L. (2010). Predictors of chronic disease at midlife and beyond – the health risks of obesity. *Maturitas, 65*, 106–111.

Palanuwech, J., Potineni, R., Roberts, R. F., & Coupland, J. N. (2003). A method to determine free fat in emulsions. *Food Hydrocolloids, 17*, 55–62.

Saberi, A. H., Chin-Ping, T., & Oi-Ming, L. (2011). Phase behavior of palm oil in blends with palm-based diacylglycerol. *Journal of the American Oil Chemists Society, 88*, 1857–1865.

Saberi, A. H., Kee, B. B., Oi-Ming, L., & Miskandar, M. S. (2011). Physico-chemical properties of various palm-based diacylglycerol oils in comparison with their corresponding palm-based oils. *Food Chemistry, 127*, 1031–1038.

Saito, S., Tomonobu, K., Hase, T., & Tokimitsu, I. (2006). Effects of diacylglycerol on postprandial energy expenditure and respiratory quotient in healthy subjects. *Nutrition, 22*, 30–35.

Saitou, K., Mitsui, Y., Shimizu, M., Kudo, N., Katsuragi, Y., & Sato, K. (2012). Crystallization behavior of diacylglycerol-rich oils produced from rapeseed oil. *Journal of the American Oil Chemists Society, 89*, 1231–1239.

Sakuno, M. M., Matsumoto, S., Kawai, S., Taihei, K., & Matsumura, Y. (2008). Adsorption and structural change of β-lactoglobulin at the diacylglycerol-water interface. *Langmuir, 24*, 11483–11488.

Shimada, A., & Ohashi, K. (2003). Interfacial and emulsifying properties of diacylglycerol. *Food Science and Technology Research, 9*, 142–147.

Srinivasan, M., Singh, H., & Munro, P. A. (2000). The effect of sodium chloride on the formation and stability of sodium caseinate emulsions. *Food Hydrocolloids, 14*, 497–507.

Tan, C. P., & Man, Y. B. C. (2000). Differential scanning calorimetric analysis of edible oils: Comparison of thermal properties and chemical composition. *Journal of the American Oil Chemists Society, 77*, 143–155.

Wang, L. J., Xie, H. E., Qiao, X. Y., Goffin, A., Hodgkinson, T., Yuan, X. F., et al. (2012). Interfacial rheology of natural silk fibroin at air/water and oil/water interfaces. *Langmuir, 28*, 459–467.

Wang, Y., Zhao, M. M., Song, K. K., Wang, L. L., Tang, S. Z., & Riley, W. W. (2010). Partial hydrolysis of soybean oil by phospholipase A_1 (Lecitase Ultra). *Food Chemistry, 121*, 1066–1072.

Waraho, T., Cardenia, V., Nishino, Y., Seneviratne, K. N., Rodriguez-Estrada, M. T., McClements, D. J., et al. (2012). Antioxidant effects of mono- and diacylglycerols in non-stripped and stripped soybean oil-in-water emulsions. *Food Research International, 48*, 353–358.

Yoshida, H., Hirakawa, Y., Tomiyama, Y., Nagamizu, T., & Mizushina, Y. (2005). Fatty acid distributions of triacylglycerols and phospholipids in peanut seeds (*Arachis hypogaea* L.) following microwave treatment. *Journal of Food Composition and Analysis, 18*, 3–14.

Young, T. (1996). Peanut oil. In Y. H. Hui (Ed.), *Bailey's industrial oil and fat products* (5th ed., pp. 377–392). New York: John Wiley & Sons.

Zhong, N., Li, L., Xu, X., Cheong, L., Li, B., Hu, S., et al. (2009). An efficient binary solvent mixture for monoacylglycerol synthesis by enzymatic glycerolysis. *Journal of the American Oil Chemists Society, 86*, 783–789.