The Effect of Polyglycerol Esters of Fatty Acids on the Crystallization of Palm Olein

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Abstract: This research investigated the effect of polyglycerol ester of fatty acids (PGE) on the crystallization of palm olein (POL). Three PGEs were studied: two solid-state PGEs (PGE1105 and PGE1117) and one liquid-state PGE (PGE1155). The addition of 0.5-5% wt. PGEs influenced the crystallization kinetics of POL and this depended on the type and concentration of the emulsifiers. During cooling down with a cooling rate of 5°C/min, the samples containing PGE1105 and PGE1117 started to crystallize at higher temperatures when compared with POL but the crystallization began at lower temperatures for the samples containing PGE1155. All samples with added PGEs exhibited lower solid fat content than that of POL after 12 h of crystallization time. The number of crystals decreased with an increase in the crystal size with PGE addition but there was no effect on polymorphism. Overall, the results suggested that PGE1105 and PGE1117 enhanced the early stages of POL crystallization possibly via the template effects but suppressed the later stages, whereas PGE1155 delayed the whole process of POL crystallization. The application of POL is often limited by its tendency to get cloudy at low temperatures during long-term storage. Based on the results, 1-5% wt. PGE1155 could be used to delay or prevent the crystallization of POL at low temperatures.

Key words: crystallization, palm olein, polyglycerol ester of fatty acids

1 Introduction

Palm oil (PO) is derived from the mesocarp of the palm fruit and is widely used in food applications. It is semi-solid at room temperature (25°C) and contains similar levels of saturated and unsaturated fatty acids. Generally, PO presents the crystal habit in β' -form, which is usually the most functional polymorph in many fat-based products¹⁾. This characteristic combined with other properties such as plasticity, oxidative stability and *trans*-fat free ensure its beneficial applications in food products²⁾. PO may be fractionated into a liquid fraction (palm olein: POL) and a more solid fraction (palm stearin: PS). POL appears clear at ambient temperature of 25°C due to its high content of C_{18:1} (39–45%) and C_{18:2} (10–13%)³⁾ and has been identified as a good ingredient for several food formulations. However, its application is often limited by its tendency to form granular crystals and get cloudy at low temperature during long-term storage. The visual observation of these crystals is usually seen as a defect by consumers although there is no deterioration in the oil quality⁴⁾.

The study of the effects of emulsifiers on the crystalliza-

tion of fat systems is of great interest for improving the quality of high fat food products such as chocolate, margarine, spreads, confectionery, bakery and dairy products⁵⁾. It is known that emulsifiers can modify the crystallization behavior of fats by either promoting or delaying the crystallization^{2,6–8)}. The emulsifiers can interact with the nucleation, crystal growth, and/or polymorphic transitions of fats depending on the miscibility of the emulsifiers and the fats⁹⁾. The influence of emulsifiers on fat crystallization could also be related to the chemical structure of triacylglycerols (TAGs) in the fat and the emulsifiers where the similarity between their structures leads to co-crystallization and the structural dissimilarity may delay the nucleation and possibly inhibit the crystal growth^{1,10,11)}. In addition, emulsifiers can act as heteronuclei, which accelerated the crystallization by direct catalytic action as impurities or seeding effect. The rate of crystal growth can be altered as a consequence of adsorption of the emulsifiers on the fat crystal surface or inclusion in the fat crystals¹²⁾.

Polyglycerol ester of fatty acids (PGE) are a broad class of food emulsifiers which are produced by polymerization

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of glycerol in the presence of an alkaline catalyst followed by esterification with fatty acids¹³. The advantageous properties of PGEs are derived from simple modification of their hydrophobicity and hydrophilicity by varying the degree of polymerization of glycerol and esterification with fatty acid moieties¹⁴. This gives access to a wide range of HLB values from 3 to 14, popularly used in many different food and nonfood applications¹⁵. PGE can be used as a crystallization modifier as they can influence nucleation and crystal growth and modify polymorphic transformation. Petrucci and Añón¹⁶ reported that a PGE rich in C₁₆, C₁₈ and C_{18:1} (~74% in total) increased the number of sunflower wax crystals in sunflower oil but decreased the crystal size. Sakamoto *et al.*¹⁷ revealed that polyglycerol behenic acid ester significantly affected the crystallization properties of PO. With the addition of 1% wt., the size of PO crystals decreased with an increase in the crystal number, indicating that polyglycerol behenic ester promoted nucleation and inhibited crystal growth of PO. In addition, the XRD patterns showed that, without the PGE, PO crystallized in the α form after rapid quenching to 10°C and transformed to the β' and β forms during heating at around 15 and 40°C, respectively. In contrast, with the PGE, PO crystallized in β' form at 10°C and did not transform to β -form during heating. Hamada *et al.*¹⁸ reported that a PGE rich in long chain fatty acids (FA) (C₁₆-C₂₂, ~60% in total) and medium chain FA (C₈-C₁₄, ~40% in total) decreased the crystallization temperature and suppressed the crystal growth of PO-based biodiesel fuels. A more recent study by Saitou *et al.*¹⁹ showed that the FA composition of PGE is important in controlling the initial step of crystallization. The addition of 0.2% wt. PGE containing C₁₆ and C_{18:1} effectively retarded the crystallization in the diacylglycerol-rich oil whereas the same amount of PGE containing only C_{18:1} did not. The retardation of crystallization may be caused by molecular interactions between fats and additives, which occur during the cluster formation processes so that cluster formation is prohibited¹¹.

This research investigated the effect of different types of PGEs (PGE1105, PGE1117 and PGE1155) on the crystallization properties (crystallization kinetics, morphology and polymorphism) of POL. The main objective of this work was to find PGE that could delay the crystallization of POL for industrial use.

2 Materials and Methods

2.1 Materials

POL (IV = 54.6 g I₂/100 g) was kindly supplied by P.P. Oil and Foods Co., Ltd. (Thailand), and was composed mainly of C_{18:1} (41.3%), C₁₆ (38.3%) and C_{18:2} (11.5%). Three types of PGEs (PGE1105, PGE1117 and PGE1155) were obtained from Palsgaard A/S, Denmark and used without future pu-

rification. PGE1105 was composed mainly of C₁₆ (45.1%), C_{18:1} (39.3%), C_{18:2} (7.6%) and C₁₈ (5.1%) and contained 52.4% of saturated FA in total. The main FA in PGE1117 were C_{18:2} (41.7%), C_{18:1} (22.8%), C₁₆ (20.8%) and C₁₈ (12.2%) with 64.8% of unsaturated FA in total whereas PGE1155 contained highest content of unsaturated FA (> 89.3%) and was composed mainly of C_{18:1} (81.5%), followed by C_{18:2} (7.1%), C₁₆ (5.1%) and C₁₈ (3.6%). PGE1105 and PGE1117 were defined as solid-state surfactants given their paste-like appearance at room temperature, whereas PGE1155 was considered a liquid-state surfactant given that it is a viscous liquid at room temperature. Blends were prepared by adding 0.5-5% wt. PGEs to POL and stirred with a magnetic stirrer at 80°C until a homogenous sample was obtained.

2.2 Crystallization and thermal profiles

The crystallization and melting profiles of the samples were analyzed with the Perkin-Elmer differential scanning calorimeter (DSC) (Model D8000, Perkin-Elmer Co., Shelton, CT, USA) following AOCS method Cj 1-94²⁰. The instrument was calibrated with indium (mp 156.6°C) as a reference standard. A sample of 3-5 mg was placed in an aluminum pan (20 μ L capacity) and hermetically sealed with a sample press. An empty pan served as reference. The samples were heated from 25 to 80°C and held for 10 min to remove any memory effect. Then they were cooled at 5°C/min to -60°C and held for 30 min followed by heating at 5°C/min to 80°C. The crystallization and melting profiles were generated during the cooling and heating, respectively. The profiles were analyzed by the software provided with the DSC.

2.3 Isothermal crystallization

The isothermal crystallization at 10°C of the samples was studied by using a pulsed-nuclear magnetic resonance (p-NMR) spectrometer (Minispec-mq20, Bruker, Karlsruhe, Germany) to follow changes in solid fat content (SFC) as a function of time. The melted samples were poured into 10 mm O.D. p-NMR tubes to a height of ~4 cm, heated at 80°C for 10 min and then transferred to a cooling bath set at 8°C. Once the sample temperature reached 10.5°C, the tube was removed from the cooling bath wiped dry and rapidly put into the p-NMR sample port set at 10 $\pm$ 0.5°C. The cooling rate was ~20°C/min. The SFC was then continuously recorded for 12 h.

The SFC data were fitted to Avrami equation²¹:

$$-\ln(1-X) = kt^n \quad (1)$$

where, t is time, k is the Avrami rate constant, X is the fractional extent of crystallization at a given time t , and n is the Avrami exponent, which represents the dimensionality of growth and type of nucleation. X was taken as the SFC at any given time normalized by the equilibrium SFC

(SFC_{eq}) obtained at the end of the crystallization time²². Curve fitting was performed after subtracting the induction time for onset of crystallization²³.

In addition, the SFC data were also fitted to the Gompertz equation²⁴:

$$F(t) = Ae^{-e\left(\frac{\mu e(\tau-t)}{A} + 1\right)} \quad (2)$$

where $F(t)$ fractional extent of crystallization at a given time t , A is the maximum fractional extent of crystallization when t approaches infinity, μ is the maximum crystallization rate and τ is the crystallization induction time. Estimation of the Gompertz parameters was performed on the basis of experimental data by non-linear regression and the parameters were used to describe the crystallization kinetics of the samples²⁵.

2.4 Crystal microstructure

The microstructure of the samples was observed by using polarized light microscopy (PLM) (Olympus BX51, Olympus Optical Co., Ltd., Tokyo, Japan) equipped with a digital camera (Olympus C-7070, Olympus Optical Co., Ltd., Tokyo, Japan). All samples were melted at 80°C for 10 min and 20 µL of each molten sample was placed on the pre-heated slide and covered by the cover slip. Then, the samples were stored at 10 ± 0.5°C for 24 h in an incubator. The crystal morphology was imaged by a 10 × lens. The sample temperature was maintained at 10°C using Peltier temperature control stage for microscopes (T95-PE Peltier System, Linkam Scientific Instruments Ltd, UK) while the images of the samples were being taken. The Image J software was used to determine the crystal size and the crystallized area of the samples.

2.5 Polymorphism

The polymorphic structure of the samples was determined by an x-ray diffractometer (XRD) (MiniFlexII, Rigaku, Japan). The samples were melted at 80°C for 10 min and poured into rectangular plastic moulds (20 mm × 25 mm × 3 mm) and then crystallized at 10 ± 0.5°C for 24 h in an incubator before XRD analysis. Scans were made in wide-angle x-ray scattering (WAXS) from 15° 2θ to 30° 2θ with a scan rate and a step width of 2° 2θ/min and 0.02° 2θ, respectively.

2.6 Statistical analysis

All experiments were performed in triplicate. The results were analyzed by analysis of variance with least significant difference (ANOVA/LSD) at 95% confidence interval.

3 Results and Discussion

3.1 Crystallization and melting thermograms

The most important aspect of the physical properties of

fats and oils is related to their crystallization and melting behavior. If the crystallization conditions changed, crystal habit, crystal size and the number of crystals could be affected and these changes will eventually be reflected in the product performance²⁶. The crystallization and melting thermograms of the bulk emulsifiers used in this work are shown in Fig. 1. PGE1105 showed a crystallization peak at 32°C and a broad melting peak at the highest peak temperature of ~38°C due to its highest content of saturated FA. PGE1117 exhibited one broad crystallization peak at ~24°C and one broad melting peak at ~36°C. PGE1155 exhibited one distinct crystallization peak at -31.9°C and one small peak at 1.4°C. Its melting profile had multiple peaks with the main one locating at -20.6°C.

The crystallization and melting thermograms of all POL + PGE samples are presented in Fig. 2. POL began to crystallize with a crystallization onset temperature (T_{CO}) of 5°C and showed one crystallization peak at 3.9°C flanked by a broad shoulder on the lower-temperature side of the peak (Fig. 2a and Table 1). The result was in line with the DSC crystallization thermogram of POL (IV = 57.7 g I₂/100 g) reported by Siew and Faridah²⁷. With the addition of 0.5-5% of PGE1105, the crystallization thermograms exhibited one main crystallization peak but with a broader characteristic than that of POL. T_{CO} of all PO + PGE1105 blends increased from that of POL ($p < 0.05$) and increased with the content of the emulsifier (Table 1), suggesting that PGE1105 induced the crystallization of the higher-melting TAGs in the fat system²⁸ due to the changes in molecular interactions between TAGs and between TAGs and the emulsifier¹⁹. The increase in T_{CO} by the addition of the solid emulsifier was in agreement with previous studies. Basso *et al.*²⁹ reported an increase in T_{CO} of PO with the addition of tripalmitin and monoacylglycerols and Domingues *et al.*¹ mentioned that T_{CO} of PO increased with the addition of sorbitan tristearate and sucrose ester (S370). In addition,

Fig. 1 DSC thermograms for crystallization and melting of bulk emulsifiers.

Fig. 2 DSC thermograms for (a) crystallization and (b) melting of POL and its mixtures with different PGEs.

Table 1 Crystallization and melting temperatures and enthalpies for POL with/without the addition of different PGEs.

Samples	Crystallization			Melting				
	T_{co} (°C)	T_c	ΔH_c (J/g)	T_{M1}	T_{M2}	T_{M3}	T_{MC} (°C)	ΔH_M (J/g)
POL	5.0 ± 0.17^e	3.9 ± 0.02^a	42.2 ± 0.21^a		6.8 ± 0.00^a	12.3 ± 0.10^a	20.2 ± 0.11^{de}	59.4 ± 0.14^{de}
POL + 0.5% PGE1105	5.5 ± 0.11^c	3.4 ± 0.35^b	33.8 ± 0.14^j	5.0 ± 0.05^a	7.2 ± 0.14^a	11.3 ± 0.04^{cde}	20.2 ± 0.08^{de}	57.6 ± 0.20^{fg}
POL + 1.0% PGE1105	5.6 ± 0.09^{bc}	3.5 ± 0.40^b	36.5 ± 0.20^{de}	4.9 ± 0.14^{ab}	7.2 ± 0.15^a	11.6 ± 0.09^{bcde}	20.5 ± 0.19^{bc}	57.3 ± 0.25^{fg}
POL + 2.0% PGE1105	5.6 ± 0.11^b	3.5 ± 0.44^b	36.6 ± 0.19^d	4.9 ± 0.10^{abc}	7.0 ± 0.19^a	11.6 ± 0.17^{bcde}	20.6 ± 0.11^b	59.4 ± 0.31^{de}
POL + 3.0% PGE1105	5.7 ± 0.19^{ab}	3.4 ± 0.40^b	37.2 ± 0.11^c	4.8 ± 0.01^{ab}	7.1 ± 0.15^a	11.7 ± 0.06^{bcd}	20.5 ± 0.10^{bc}	62.5 ± 0.24^{bc}
POL + 5.0% PGE1105	5.8 ± 0.12^a	3.4 ± 0.38^b	41.6 ± 0.14^b	4.8 ± 0.05^{abc}	6.9 ± 0.08^a	11.9 ± 0.03^{ab}	20.5 ± 0.08^{bc}	84.2 ± 0.24^a
POL + 0.5% PGE1117	5.2 ± 0.06^d	3.1 ± 0.06^b	36.0 ± 0.21^f	4.6 ± 0.01^{bc}	7.0 ± 0.18^a	11.5 ± 0.31^{cde}	20.3 ± 0.13^{de}	64.0 ± 0.19^b
POL + 1.0% PGE1117	5.3 ± 0.12^d	3.1 ± 0.25^b	36.1 ± 0.13^f	4.6 ± 0.06^{bc}	7.0 ± 0.47^a	11.2 ± 0.19^e	20.4 ± 0.07^{cd}	62.5 ± 0.26^{bc}
POL + 2.0% PGE1117	5.5 ± 0.18^c	3.1 ± 0.05^b	35.5 ± 0.22^h	4.8 ± 0.08^{abc}	7.1 ± 0.33^a	11.2 ± 0.08^e	20.3 ± 0.11^{cd}	59.8 ± 0.23^d
POL + 3.0% PGE1117	5.5 ± 0.08^c	2.9 ± 0.00^b	34.8 ± 0.19^i	4.8 ± 0.19^{bc}	7.0 ± 0.15^a	11.4 ± 0.25^{de}	20.3 ± 0.07^d	59.4 ± 0.41^{de}
POL + 5.0% PGE1117	5.5 ± 0.14^c	2.7 ± 0.02^b	33.4 ± 0.15^k	4.8 ± 0.02^{ab}	6.9 ± 0.02^a	11.5 ± 0.22^{abc}	21.0 ± 0.14^a	58.4 ± 0.31^{def}
POL + 0.5% PGE1155	4.9 ± 0.13^f	4.1 ± 0.00^a	36.4 ± 0.21^e	4.8 ± 0.06^{ab}	7.1 ± 0.01^a	11.7 ± 0.03^{abc}	20.1 ± 0.16^{de}	62.2 ± 0.17^{bc}
POL + 1.0% PGE1155	4.8 ± 0.06^f	3.9 ± 0.18^a	35.8 ± 0.11^g	4.7 ± 0.09^{abc}	7.1 ± 0.00^a	11.7 ± 0.14^{abc}	20.1 ± 0.12^e	61.6 ± 0.30^c
POL + 2.0% PGE1155	4.9 ± 0.11^f	4.0 ± 0.10^a	35.0 ± 0.16^i	4.6 ± 0.00^{bc}	6.8 ± 0.02^a	11.8 ± 0.01^{ab}	20.2 ± 0.10^{de}	56.8 ± 0.27^g
POL + 3.0% PGE1155	4.8 ± 0.08^f	4.1 ± 0.02^a	33.6 ± 0.09^k	4.6 ± 0.04^c	6.6 ± 0.02^a	11.8 ± 0.09^{ab}	20.2 ± 0.08^{de}	57.1 ± 0.21^{fg}
POL + 5.0% PGE1155	4.8 ± 0.15^f	3.9 ± 0.10^a	33.4 ± 0.14^k	4.7 ± 0.02^{abc}	6.6 ± 0.04^a	11.7 ± 0.15^{abc}	20.2 ± 0.14^{de}	56.2 ± 0.19^g

Note: For crystallization, T_c is the crystallization peak temperature and T_{co} is the crystallization onset temperature. For melting, T_{M1} is the melting temperature for the melting peak at lowest temperature and T_{M3} is the melting temperature for the melting peak at highest temperature. T_{MC} is the melting completion temperature. ΔH_c and ΔH_M are the enthalpies of crystallization and melting, respectively. Different letters in the same column are significantly different ($p < 0.05$).

the sample with 5% PGE1105 exhibited a small exothermic peak at $\sim 21^\circ\text{C}$ (arrow a) which could represent the crystallization of the emulsifier^{11, 30)} or a segregation of the high-melting TAGs of POL due to the interactions among TAGs and PGE1105²⁸⁾. Domingues *et al.*¹⁾ showed similar results in relation to the isolated crystallization of S370 at high temperature, accelerating the crystallization of TAGs with high melting point in PO. Verstringe *et al.*³⁰⁾ suggested that high-melting monoacylglycerols can initiate the crystallization process and induce fractional crystallization of high-melting TAGs in PO probably with crystallized monoacylglycerols acting as template. The addition of PGE1117 had a similar effect on the crystallization thermogram of POL as PGE1105. The addition of PGE1155 at all concentrations resulted in similar crystallization profiles to POL, but with a decrease in T_{CO} ($p < 0.05$), indicating that PGE1155 delayed POL crystallization. The crystallization enthalpy (ΔH_{C}) of POL was $\sim 42 \text{ J/g}$ and this value decreased significantly with the addition of all PGEs ($p < 0.05$) with the largest divergence seen with the PGE1155, especially at high concentrations.

The melting thermograms of POL exhibited multiple endothermic peaks (Fig. 2b) relating to the melting of different groups of TAGs, indicating that there were several types of homogeneous polycrystalline of POL¹²⁾. The two main melting peaks of POL were at $\sim 7^\circ\text{C}$ and $\sim 12^\circ\text{C}$, representing the melting of low- and high-melting fractions. The addition of all PGEs at 0.5–5% altered the melting thermogram slightly from that of POL with an additional melting peak at $\sim 5^\circ\text{C}$ (arrow b). The peak was more prominent with the addition of PGE1105 and PGE1117 compared to PGE1155. In general, the melting completion temperatures (T_{MC}) were not significantly affected by the addition of the three surfactants ($p > 0.05$) (Table 1). The melting enthalpies (ΔH_{M}) of POL + PGE1105 blends increased as the content of the emulsifier in the blends increased ($p < 0.05$), indicating the more ordered arrangement of POL TAGs. In contrast, ΔH_{M} of POL + PGE1117 blends and POL + PGE1155 blends decreased as their content in the blends increased ($p < 0.05$).

3.2 Isothermal crystallization

The isothermal crystallization curves of POL and POL + PGE blends at 10°C are given in Fig. 3. The shape of the crystallization curve, which is an SFC versus time curve, can give important clues for the crystallization mechanism of a particular fat system. POL started to crystallize at $\sim 0.5 \text{ h}$ and showed two-step crystallization with an initial SFC rise to $\sim 2\%$ within $\sim 1.5 \text{ h}$ (step 1) followed by a rapid SFC rise starting at $\sim 3 \text{ h}$ before reaching an equilibrium SFC (SFC_{eq}) of $\sim 38\%$ after 12 h (step 2) (Table 2). This was consistent with what was observed by Zhang *et al.*²⁶⁾ during isothermal crystallization of POL at 10°C .

The crystallization curves of POL + PGEs were different

Fig. 3 Crystallization curves of POL and its mixture with (a) PGE1105, (b) PGE1117 and (c) PGE1155 obtained during isothermal crystallization at 10°C .

from that of POL, an indication of different crystallization mechanisms. PGE1105 did not alter the two-step crystallization pathway of POL (Fig. 3a). However, the samples began to crystallize before the temperature reached 10°C during fast cooling with no induction time, suggesting that PGE1105 accelerated the early stages of POL crystalliza-

Table 2 Equilibrium solid fat content (SFC_{eq}) of POL with/without the addition of different PGEs after isothermal crystallization at 10°C for 12 h.

Samples	SFC _{eq} (%)		
	PGE1105	PGE1117	PGE1155
POL	38.3 ± 0.08 ^a	38.3 ± 0.08 ^a	38.3 ± 0.08 ^a
POL + 0.5% PGE	37.0 ± 0.11 ^b	35.7 ± 0.12 ^{cd}	35.3 ± 0.09 ^d
POL + 1.0% PGE	36.4 ± 0.14 ^{bc}	35.9 ± 0.08 ^{cd}	33.0 ± 0.16 ^e
POL + 2.0% PGE	36.0 ± 0.10 ^{bcd}	35.5 ± 0.05 ^{cd}	29.7 ± 0.03 ^f
POL + 3.0% PGE	35.3 ± 0.11 ^d	33.0 ± 0.11 ^e	27.7 ± 0.11 ^g
POL + 5.0% PGE	33.4 ± 0.08 ^e	30.4 ± 0.17 ^f	25.8 ± 0.12 ^h

Mean values followed by different letters are significantly different ($p < 0.05$).

Table 3 Avrami rate constant (k) and Avrami exponent (n) for the crystallization at 10°C of POL with/without the addition of different PGEs ($R^2 > 0.999$).

Samples	k (min ⁻¹)	n
POL	0.040 ± 0.21 ^g	2.47 ± 0.06 ^a
POL+0.5% PGE1105	0.435 ± 0.16 ^a	2.33 ± 0.11 ^a
POL+1.0% PGE1105	0.418 ± 0.10 ^b	1.87 ± 0.10 ^{fg}
POL+2.0% PGE1105	0.338 ± 0.23 ^c	2.05 ± 0.13 ^{bcd}
POL+3.0% PGE1105	0.284 ± 0.26 ^d	1.77 ± 0.19 ^f
POL+5.0% PGE1105	0.206 ± 0.19 ^f	1.86 ± 0.09 ^{fg}
POL+0.5% PGE1117	0.334 ± 0.21 ^c	2.11 ± 0.12 ^b
POL+1.0% PGE1117	0.270 ± 0.11 ^c	1.96 ± 0.16 ^{de}
POL+2.0% PGE1117	0.217 ± 0.13 ^f	1.83 ± 0.14 ^g
POL+3.0% PGE1117	0.044 ± 0.19 ^g	1.97 ± 0.08 ^{de}
POL+5.0% PGE1117	0.034 ± 0.10 ^{gh}	2.03 ± 0.21 ^{bcd}
POL+0.5% PGE1155	0.042 ± 0.24 ^g	2.12 ± 0.19 ^a
POL+1.0% PGE1155	0.038 ± 0.14 ^{gh}	2.44 ± 0.13 ^a
POL+2.0% PGE1155	0.034 ± 0.19 ^{gh}	1.97 ± 0.21 ^{de}
POL+3.0% PGE1155	0.036 ± 0.20 ^{gh}	2.15 ± 0.16 ^{bc}
POL+5.0% PGE1155	0.025 ± 0.24 ^h	2.11 ± 0.11 ^{cde}

Different letters in the same column are significantly different ($p < 0.05$).

tion. The effect was stronger with higher content of PGE1105. The observed behavior was similar to the effect of solid-state sucrose esters (S170 and P170) on the crystallization of POL³¹⁾ and a PO-PS blend³²⁾. The same effect was also found in the crystallization of cocoa butter with the addition of solid-state sorbitan esters³³⁾. After the first step of crystallization, the SFC climbed rapidly to reach a plateau and SFC_{eq}. At the beginning of the SFC increase, there was a crossover in SFC at a time point (arrow c) after which the sample with lower content of PGE1105 showed higher SFC and *vice versa*. At the end of crystallization time, the sample with higher PGE1105 content showed

lower SFC_{eq} ($p < 0.05$) (Table 2). This result was in contrast to the effect of S170 on the SFC_{eq} of POL which increased with higher content of S170³¹⁾. It is unclear why the concentration responses were different between the emulsifiers, but this suggests that they have different mechanisms in controlling POL crystallization, probably depending on the solubility of the emulsifiers in the oil¹⁹⁾.

The crystallization curves of POL + PGE1117 blends demonstrated a more complex behavior than POL + PGE1105 blends (Fig. 3b). The blends containing ≤ 3% of PGE1117 started to crystallize at approximately the same time as POL (0.5 h). However, the second step of crystalli-

Table 4 Gompertz parameters for the crystallization at 10°C of POL with/without the addition of different PGEs ($R^2 > 0.999$).

Samples	τ	A	μ
POL	204.7 ± 0.00^{de}	38.2 ± 0.00^a	0.17 ± 0.00^d
POL+0.5% PGE1105	74.4 ± 2.99^g	35.9 ± 0.02^{bc}	0.26 ± 0.01^a
POL+1.0% PGE1105	74.7 ± 5.47^g	36.2 ± 0.05^b	0.25 ± 0.00^a
POL+2.0% PGE1105	—	—	—
POL+3.0% PGE1105	—	—	—
POL+5.0% PGE1105	—	—	—
POL+0.5% PGE1117	101.0 ± 3.49^{fg}	35.5 ± 0.03^{bcd}	0.23 ± 0.01^b
POL+1.0% PGE1117	127.8 ± 3.27^f	35.5 ± 0.03^{bc}	0.22 ± 0.00^b
POL+2.0% PGE1117	130.4 ± 3.58^f	35.3 ± 0.04^{cd}	0.19 ± 0.00^c
POL+3.0% PGE1117	177.0 ± 6.99^c	32.9 ± 0.10^g	0.14 ± 0.00^c
POL+5.0% PGE1117	266.7 ± 15.77^b	33.1 ± 0.13^{fg}	0.10 ± 0.01^g
POL+0.5% PGE1155	182.3 ± 0.00^c	35.2 ± 0.00^{bc}	0.14 ± 0.00^c
POL+1.0% PGE1155	242.9 ± 0.03^{bc}	34.6 ± 0.00^{de}	0.11 ± 0.00^f
POL+2.0% PGE1155	250.2 ± 0.02^{bc}	33.8 ± 0.01^{ef}	0.09 ± 0.00^g
POL+3.0% PGE1155	249.7 ± 0.01^{cd}	34.2 ± 0.01^c	0.07 ± 0.00^h
POL+5.0% PGE1155	308.3 ± 0.03^a	33.1 ± 0.00^{fg}	0.07 ± 0.00^h

τ : induction time (min), A: the maximum fractional extent of crystallization (%), μ : the maximum crystallization rate (%/min).

Data fitting were not performed with samples with 2%, 3% and 5% of PGE1105 due to the lack of the crystallization induction time.

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

zation of the blends with 0.5-2% occurred sooner than POL (at 1.5 h for 0.5% and at 2 h for 1-2% PGE1117). At the end of the experiments, these samples reached SFC_{eq} of $\sim 36\%$. The blend with 3% PGE1117 showed a small effect on the early stage of POL crystallization but exhibited a stronger effect on the later stage with a significantly lower SFC_{eq} of 33% ($p < 0.05$). In contrast, the blends with 5% of PGE1117, although began the first step crystallization before the temperature reached 10°C, drastically delayed the second step crystallization until 4 h with a significant drop in SFC_{eq} (30%) at the end of the experiment compared to POL ($p < 0.05$).

The addition of PGE1155 at all concentrations did not affect the first step of POL crystallization (Fig. 3c). However, the second step of POL crystallization was significantly delayed by all concentrations of PGE1155, except 0.5%. At the end of the experiments, all samples exhibited the SFC_{eq} significantly lower than that of POL ($p < 0.05$). All POL + PGE blends showed lower SFC_{eq} than that of POL, suggesting that all PGEs suppressed the later stages of POL crystallization with varying degree. The interaction of the fatty acids of the TAGs and the acyl groups of the emulsifiers was selective and led to different rates of retar-

dation and acceleration of crystallization³⁴⁾.

The SFC crystallization curves were fitted to Avrami model via linear regression. The Avrami rate constant (k) and Avrami exponent (n) for the crystallization of POL at 10°C were 0.04 and 2.47, respectively (Table 3). With the addition of PGE1105 and PGE1117, the k value significantly increased from that of POL ($p < 0.05$), indicating the increase in the driving force for crystallization. The k values for POL + PGE1105 blends decreased from 0.435 to 0.206 ($p < 0.05$) and the k values for POL + PGE1117 decreased from 0.334 to 0.034 ($p < 0.05$) when the concentration of both emulsifiers increased from 0.5 to 5%. In contrast, the k values for all POL + PGE1155 blends, except the blend with 0.5% PGE1155, were lower than that of POL ($p > 0.05$) and the k value decreased as the content of PGE1155 in the blend increased, suggesting that the crystallization of POL was suppressed by PGE1155. The n values of all POL + PGE blends were between 1.8 and 2.4. The n values can be associated with the mechanisms of crystal growth and fractional numbers for n values indicate the contribution of more than one crystallization mechanism²³⁾.

The SFC crystallization curves fitted to the Gompertz equation revealed that the crystallization kinetics of POL

were significantly influenced by the addition of PGEs (Table 4). The equation presents an induction time and crystallization rate in crystallization process that depend on the number of nuclei available for crystal growth²⁴⁾. Adding 0.5 and 1% PGE1105 to POL led to a reduction in the induction time with an increase in the crystallization rate ($p < 0.05$). The addition of 0.5-2% PGE1117 also resulted in a decrease in the induction time and an increase in the crystallization rate from that of POL ($p < 0.05$) but 3-5% PGE1117 decreased the crystallization rate ($p < 0.05$) whereas the addition of all concentrations of PGE1155 cause the crystallization rate to decrease significantly ($p < 0.05$). The results were in line with the data fitting with Avrami model presented above which showed that the crystallization rate generally increased with the addition of PGE1105 and PGE1117 but decreased when PGE1155 was added.

3.3 Crystal microstructure

Figure 4 presents the microstructure of POL crystallized at 10°C for 24 h with and without PGE addition. POL exhibited a large number of small spherulites measuring $13.0 \pm 3.3 \mu\text{m}$ in diameter with some degree of aggregation and a crystallized area of $60.7 \pm 0.4\%$. The result confirmed that POL can readily form a number of small crystals at low temperature (10°C), leading to the clouding problem during storage. The addition of PGEs did not affect the crystal shape of POL, with all blends showing spherulitic crystals. However, there were some changes in the crystal number and size compared to POL without additives. 0.5% PGE1105 did not have any significant effect on the microstructure of POL. As the content of PGE1105 increased

further, the crystal size increased significantly from $9.8 \pm 3.3 \mu\text{m}$ for 0.5% PGE1105 to $46.7 \pm 6.7 \mu\text{m}$ for 5% PGE1105 ($p < 0.05$) with a decrease in the crystallized area ($60.6 \pm 1.9\%$ for 0.5% PGE1105 to $43.1 \pm 1.3\%$ for 5% PGE1105, $p < 0.05$) and a reduction in the number of crystal aggregates. The addition of PGE1117 resulted in a similar trend as that with PGE1105 but with larger crystal sizes measuring 14.4 ± 4.8 and $56.5 \pm 11.6 \mu\text{m}$ in diameter for 0.5% and 5% PGE1117, respectively. The crystallized area also reduced from 55.2 ± 0.8 for 0.5% PGE1117 to $39.1 \pm 0.3\%$ for 5% PGE1117 ($p < 0.05$). The results were in agreement with a previous report by Saitou *et al.* (2014) which showed a decrease in the crystal number of diacylglycerol-rich oil with an increase in the crystal size due to an addition of PGEs, suggesting that the rate of nucleation was reduced by the addition of PGEs¹⁹⁾, but contradicted what was reported by Sakamoto *et al.* that the addition of polyglycerol behenic acid esters to PO at 20°C led to an increase in crystal number and a decrease in crystal size¹⁷⁾. The PLM results with PGE1105 and PGE1117 correlated well with the SFC results, which showed lower SFC_{eq} with increasing PGE concentration (Table 2). The presence of PGE1155 resulted in a smaller increase in the crystal size (from $13.0 \pm 3.5 \mu\text{m}$ for 0.5% PGE1155 to $28.0 \pm 5.1 \mu\text{m}$ for 5% PGE1155, $p < 0.05$) and the formation of noticeably fewer crystal aggregates. The crystallized area (~ 32 -33%) did not change significantly with an increase in the content of PGE1155 ($p > 0.05$).

3.4 Polymorphism

Figure 5 presents the x-ray diffraction patterns of POL with and without PGEs crystallized at 10°C. The XRD

Fig. 4 Crystal microstructure of POL and its mixtures with different PGEs obtained after isothermal crystallization at 10°C for 24 h.

Fig. 5 X-ray diffraction patterns of POL and its mixture with different PGEs obtained after isothermal crystallization at 10°C for 24 h.

pattern of POL exhibited two short-spacing peaks at 3.9 and 4.2 Å, indicating the occurrence of the β' form³⁵⁾. In general, PO is highly stable in the β' form due to the large amount of TAGs with 50 and 52 carbons which arrange as β' crystals²⁸⁾. The addition of PGEs did not affect the POL polymorphism. All POL + PGE blends showed the same diffraction patterns as POL with two short-spacing peaks of β' form at 3.9 and 4.2 Å. Sakamoto *et al.* reported that the addition of polyglycerol behenic acid esters to PO promote the crystallization of β' form¹⁷⁾.

This study has shown that the addition of PGEs influenced the crystallization kinetics of POL but did not affect the polymorphism. Overall, the results suggested that the solid-state PGEs (PGE1105 and PGE1117) enhanced the early stages of POL crystallization but suppressed the later stages, whereas the liquid-state PGE (PGE1155) delayed the whole process of POL crystallization. When emulsifiers begin to crystallize before the fat during cooling down, the emulsifiers could promote the crystallization of the fat via template effects¹¹⁾ which refer to the phenomenon where a higher melting additive with significant structural and chemical similarities to the lipid serves as templates (seeding nuclei) for heterogeneous crystallisation of lipids³⁶⁾. PGE1105 and PGE1117 have higher melting points than POL, hence could crystallize before POL upon cooling and increase nucleation of POL via template effects¹¹⁾. The FA composition of PGEs is important in controlling the initial step of crystallization¹⁹⁾ and the concentration <0.1% of the emulsifiers may be enough to have a strong impact on nucleation³⁷⁾. PGE1105 exhibited stronger effect during early stages of POL crystallization than PGE1117 probably due to higher degree of similarity between its fatty acid moieties (C_{16} and $C_{18:1}$) and those of POL^{34, 37)}. During the later stages of crystallization, both emulsifiers could be absorbed at the kink sites of crystallizing POL and then prohibit the incorporation of the POL TAGs through large polyglycerol groups¹⁹⁾. In contrast, PGE1155, an emulsifier

which contained the highest content of $C_{18:1}$ in this group, suppressed POL crystallization all the way. It was liquid at 10°C hence it did not crystallize before POL to give the template effects. The nucleation retardation may be related to molecular interactions between fat and additives, which occur during the cluster formation process so that the cluster formation is prohibited. The large polar polyglycerol groups of PGE1155 could prevent the POL TAGs from incorporating into clusters and nuclei due to steric hindrance caused by the difference in size of the polar groups¹⁹⁾, resulting in the delay in POL crystallization. It was also possible that PGE1155 delayed POL crystallization by preventing the clusters of the fat from forming crystal nuclei through attractive molecular interactions between the emulsifier and the fat molecules (de-clustering effect)¹¹⁾.

4 Conclusion

PGE1105, PGE1117 and PGE1155 affected the crystallization kinetics of POL and the effect depended on the type and concentration of the emulsifiers. In general, the crystallization induction time decreased and the crystallization rate increased with the addition of PGE1105 and PGE1117. The crystal number and the crystallized area decreased with an increase in the crystal size as the concentration of PGE1105 and PGE1117 in the blends increased. The addition of PGE1117 resulted in an increase in the crystallization induction time and a decrease in the crystallization rate. Overall, PGE1105 and PGE1117 accelerated the early stage possibly via the template effects but suppressed the later stages of POL crystallization. In contrast, PGE1155 suppressed POL crystallization at all time points. There was no effect on the polymorphism of POL by any of the PGEs. Based on the results, PGE1155 could be used to delay or prevent the crystallization of POL at low temperatures.

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